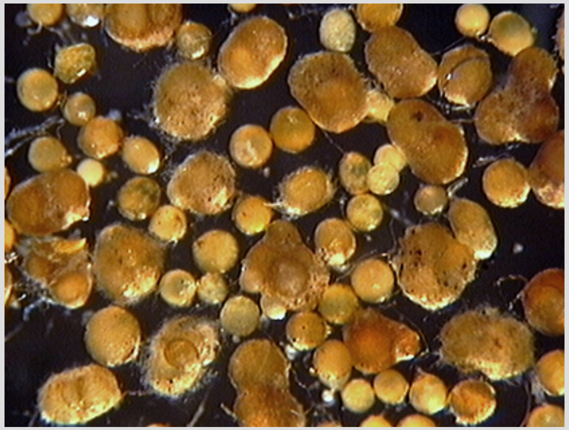
Agroecology for IPM III
Diseases



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 violoae

Root and stem rot

Phytopthora 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

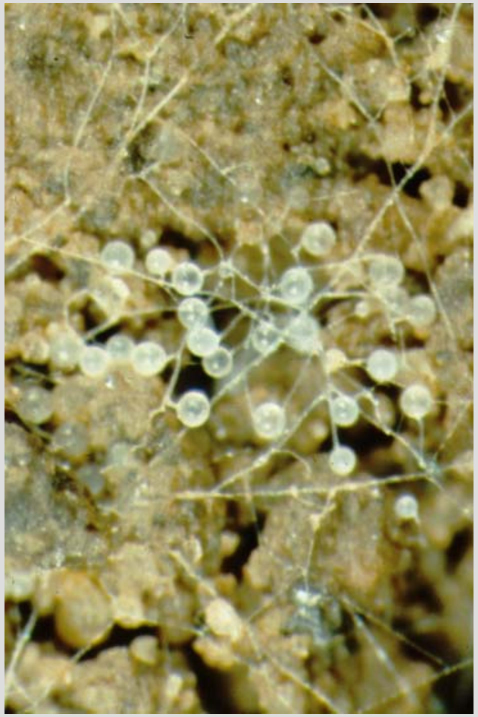
- Resistant varieties
- Mechanical control of soilborne diseases
- Thermal control of soilborne diseases
- Botanicals
- Cropping system design
- Timing of sowing

- (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)

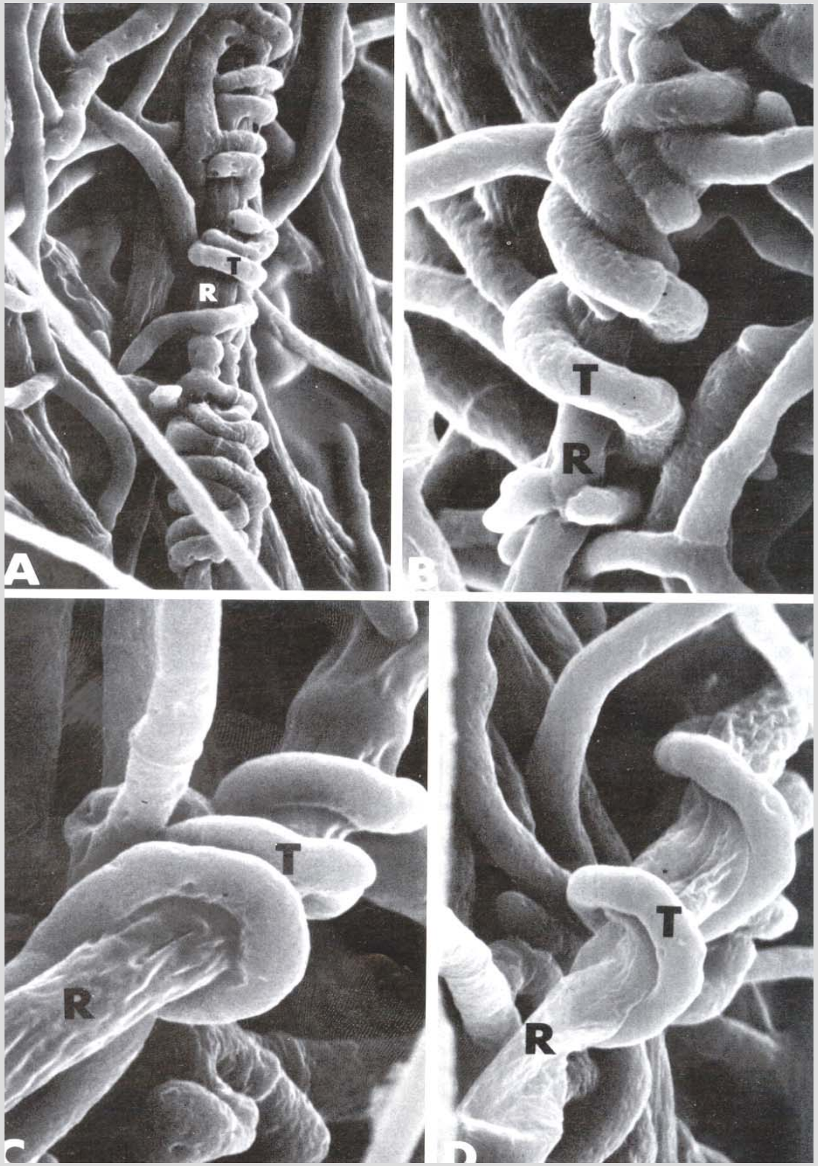
- Competition
- Antibiosis
- Parasitism
- Grazing
- Induction of plant defense

Microbial antibiotics

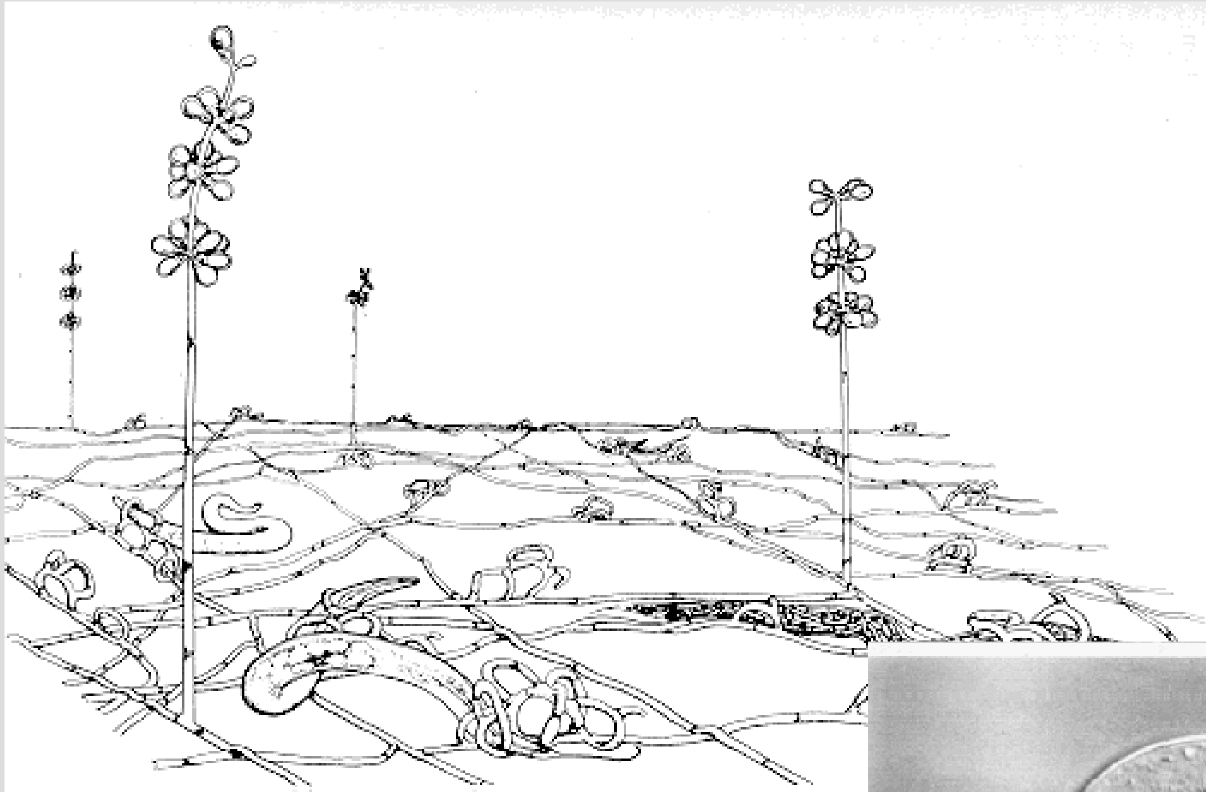
Table 2. Some of antibiotics produced by BCAs

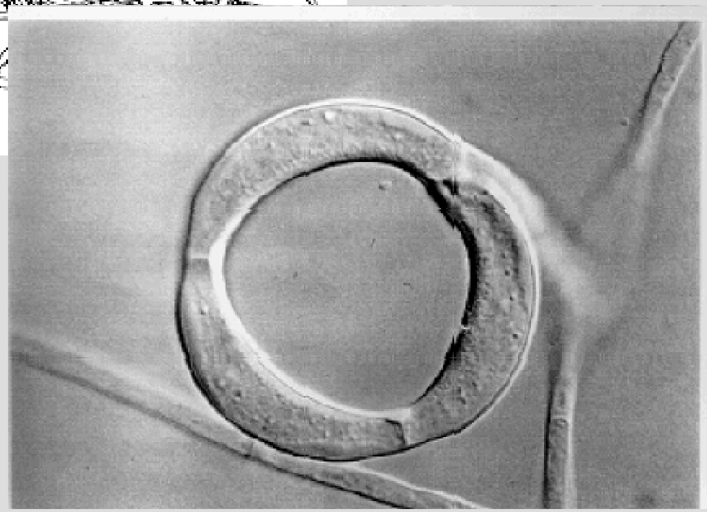
Antibiotic	Source	Target pathogen	Disease	Reference
2, 4-diacetyl-phloroglucinol	*Pseudomonas fluorescens* F113	*Pythium spp.*	Damping off	Shanahan et al. (1992),
Agrocin 84	*Agrobacterium radiobacter*	*Agrobacterium tumefaciens*	Crown gall	Kerr (1980)
Bacillomycin D	*Bacillus subtilis* AU195	*Aspergillus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

Mycoparasitism of *Rhizoctonia* by *Trichoderma*



Nematode trapping fungi





Fungal grazing by Collembola

Foto: John Larsen

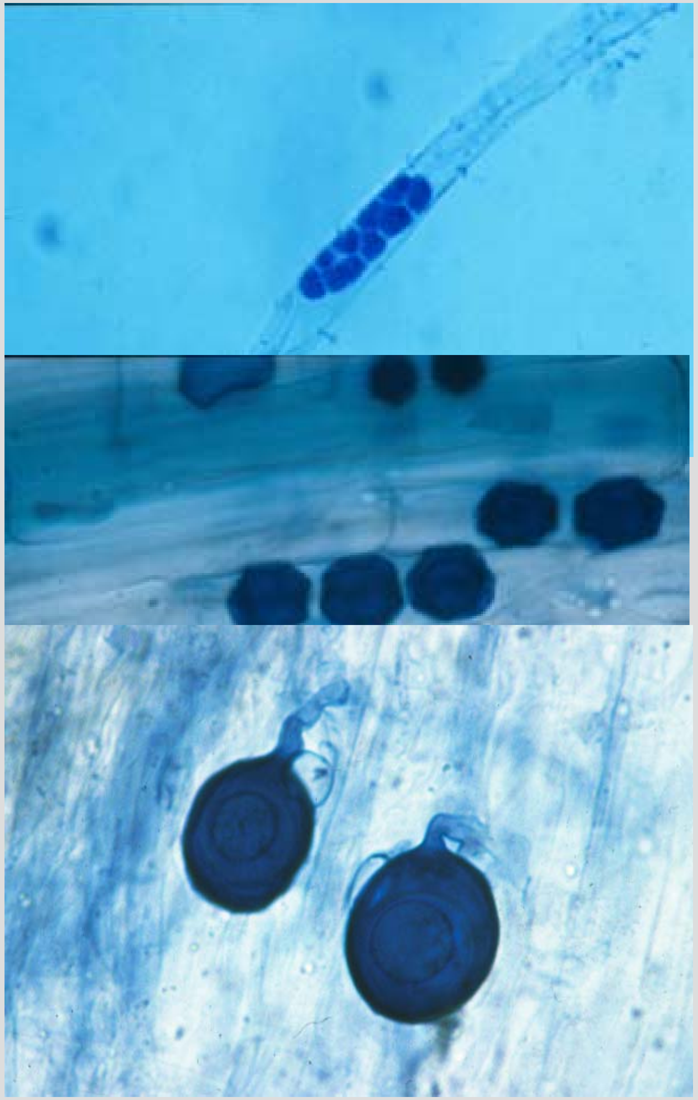
Plant defense induction

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

Bacterial strain	Plant species	Bacterial determinant	Type	Reference
Bacillus mycoides strain Bac J	Sugar beet	Peroxidase, chitinase and β-1,3-glucanase	ISR	Bargabus et al. (2002)
Bacillus pumilus 203-6	Sugar beet	Peroxidase, chitinase and β-1,3-glucanase	ISR	Bargabus et al. (2004)
Bacillus subtilis GB03 and IN937a	*Arabidopsis*	2,3-butanediol	ISR	Ryu et al. (2004)
Pseudomonas fluorescens strains				
CHA0	Tobacco	Siderophore	SAR	Maurhofer et al. (1994)
	Arabidopsis	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)
	Arabidopsis	Lipopolysaccharide	ISR	Van Wees et al. (1997)
	Tomato	Lipopolysaccharide	ISR	Duijff et al. (1997)
Pseudomonas putida strains	*Arabidopsis*	Lipopolysaccharide	ISR	Meziane et al. (2005)
WCS 358	*Arabidopsis*	Lipopolysaccharide	ISR	Meziane et al. (2005)
		Siderophore	ISR	Meziane et al. (2005)
BTP1	Bean	Z,3-hexenal	ISR	Ongena et al. (2004)
Serratia marcescens 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 allelopatic 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*

Saprotrofic fungi

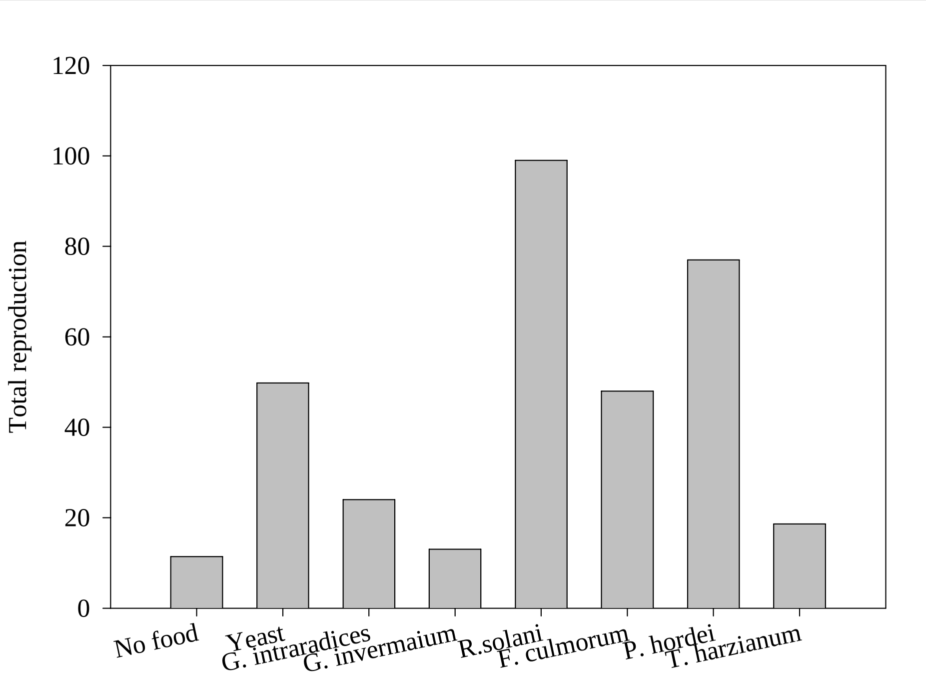
- *Pencillium hordei*
- *Trichoderma harzianum*

Mycorrhizal fungi

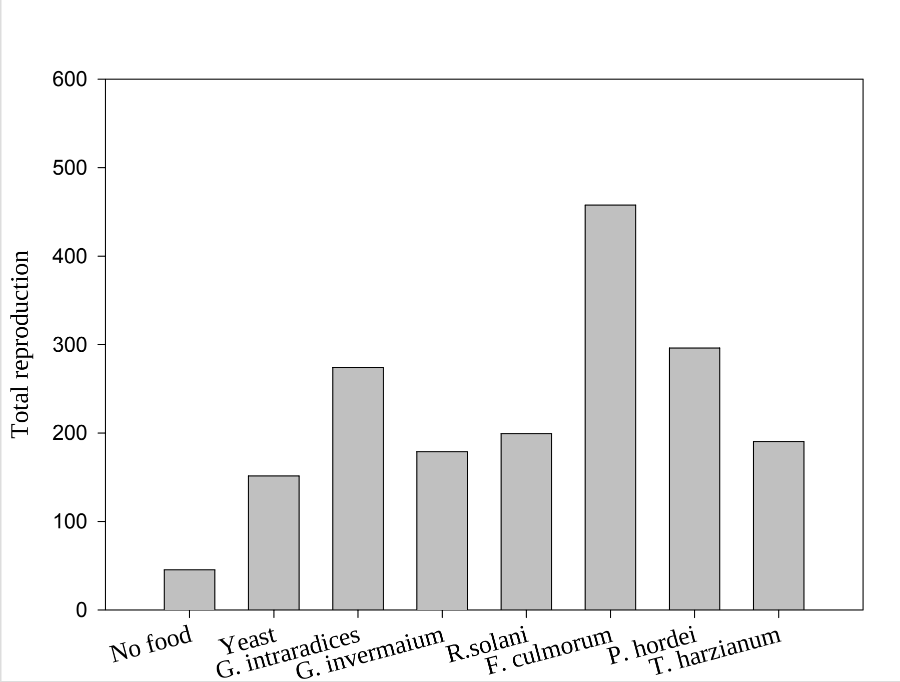
- *Glomus intraradices*
- *Glomus invermaium*

John Larsen

Collembola reproduction on different fungal food items



Folsomia fimetaria



Folsomia candida

John Larsen

Examples of foliar pathogens

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

Biocontrol of foliar diseases

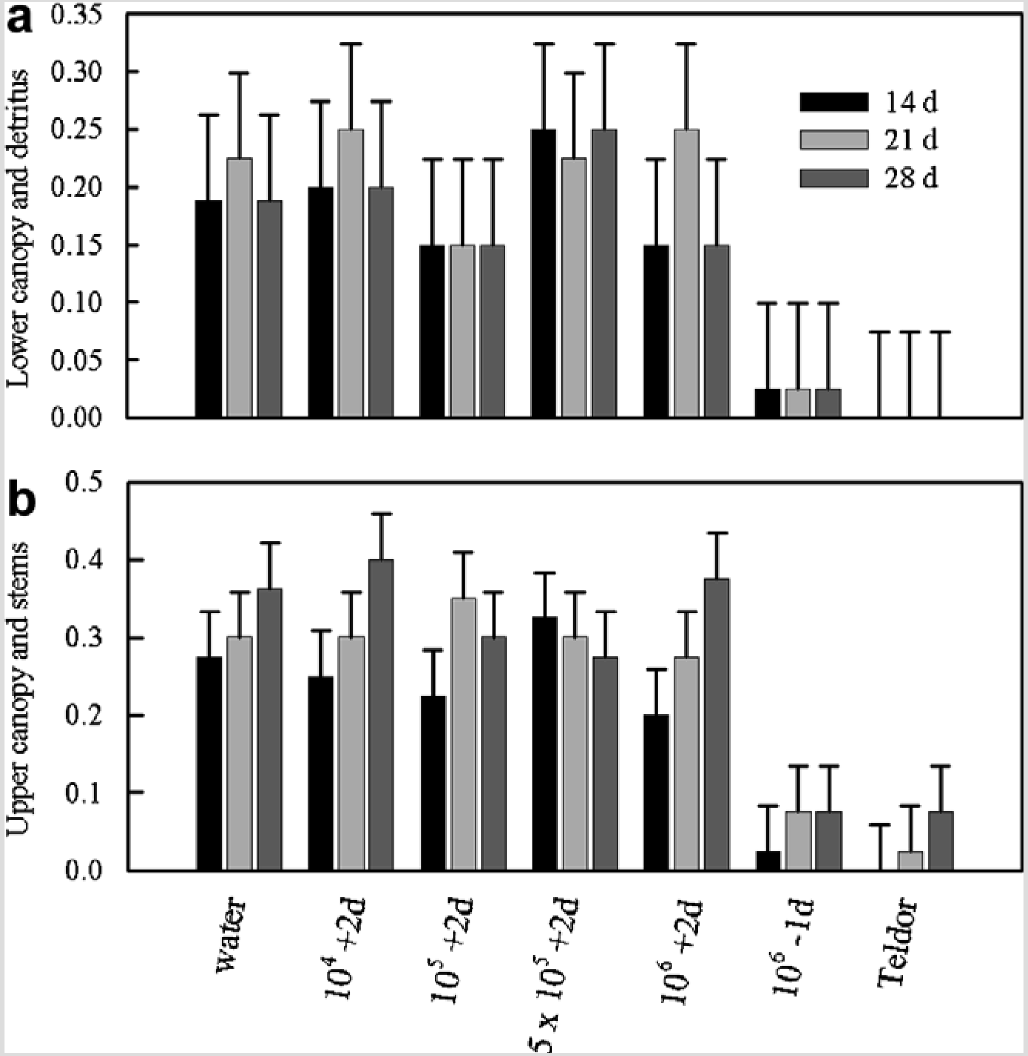
- Induction of plant defense
- BCAs applied to the foliage
- Botanicals (plant extracts)

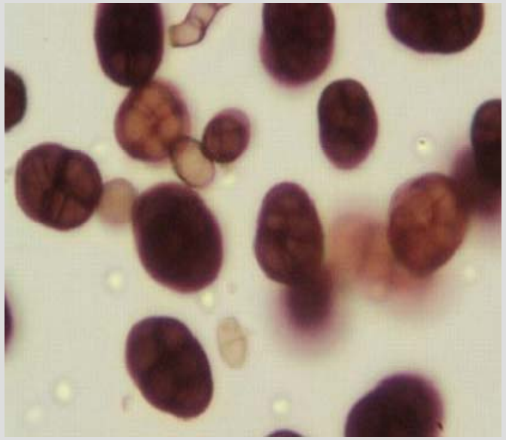
Effects of *Ulocladium atrum* against grey mold in pot roses – an example of biological control of a foliar pathogen

without *U. atrum*

with *U. atrum*

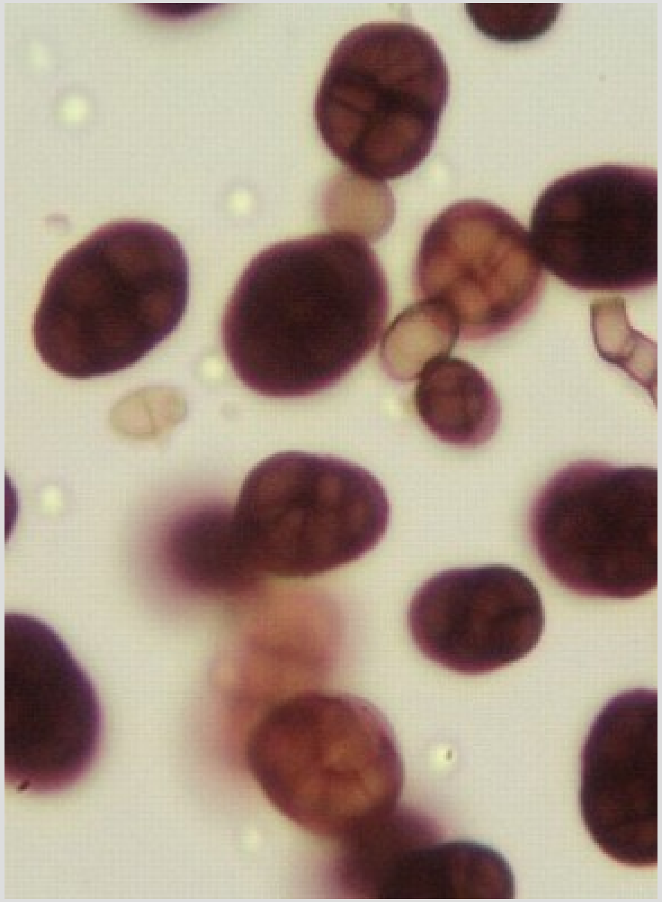
Control of grey mould in pot roses by *Ulocladium atrum*



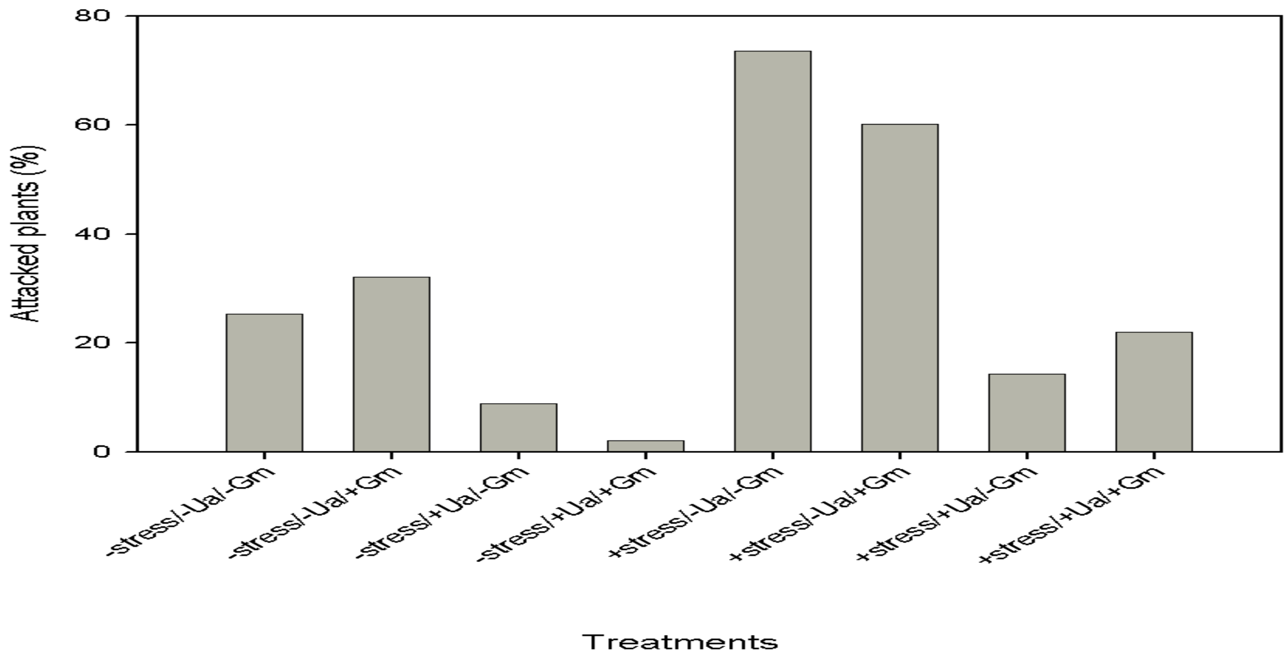


Yohalem et al, Biological Control 2007

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*



Stress, *

U. atrum, ***

Stress x *U.a* x *G.m*, *

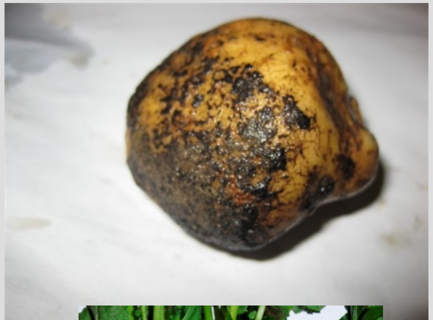
Biological management of plant diseases – a summary

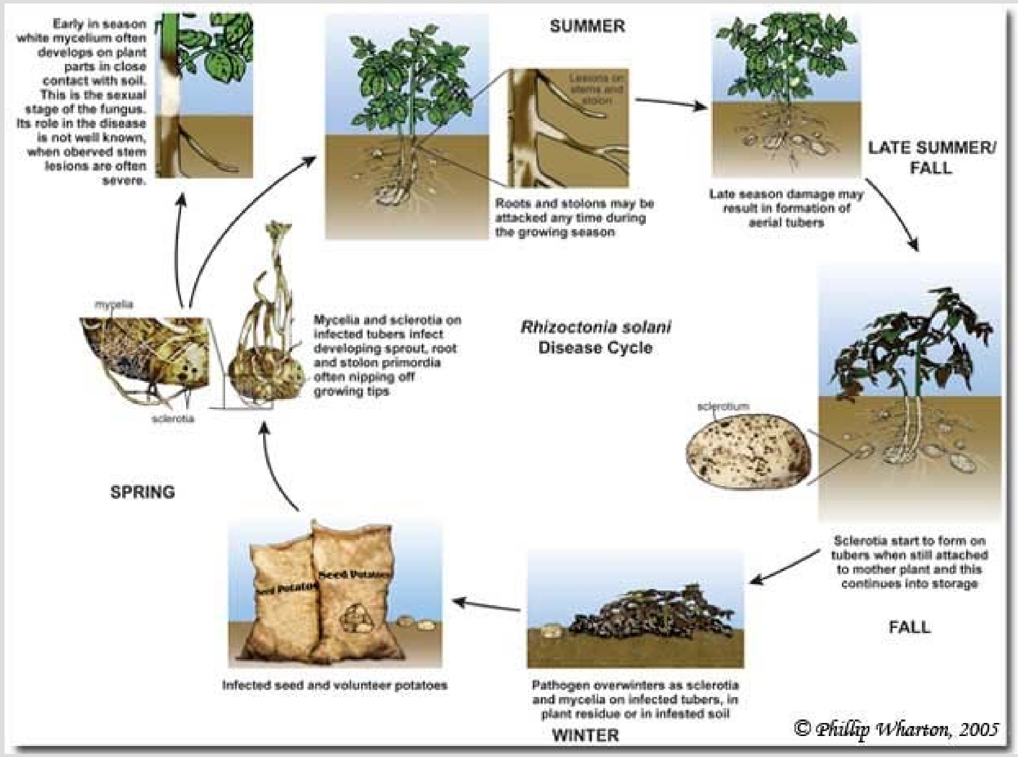
- There are several modes of actions in biological management of plant diseases, and this is important be aware of when developing agroecological IPM strategies
- Strategies to manage root- and foliar pathogens can be different
- Combination of more biological strategies to control a disease can be an 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 2007and 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 insidence

- No effects

2009 High disease insidence

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

Effects of the single factors on yield of potatoes

2008 Low disease insidence

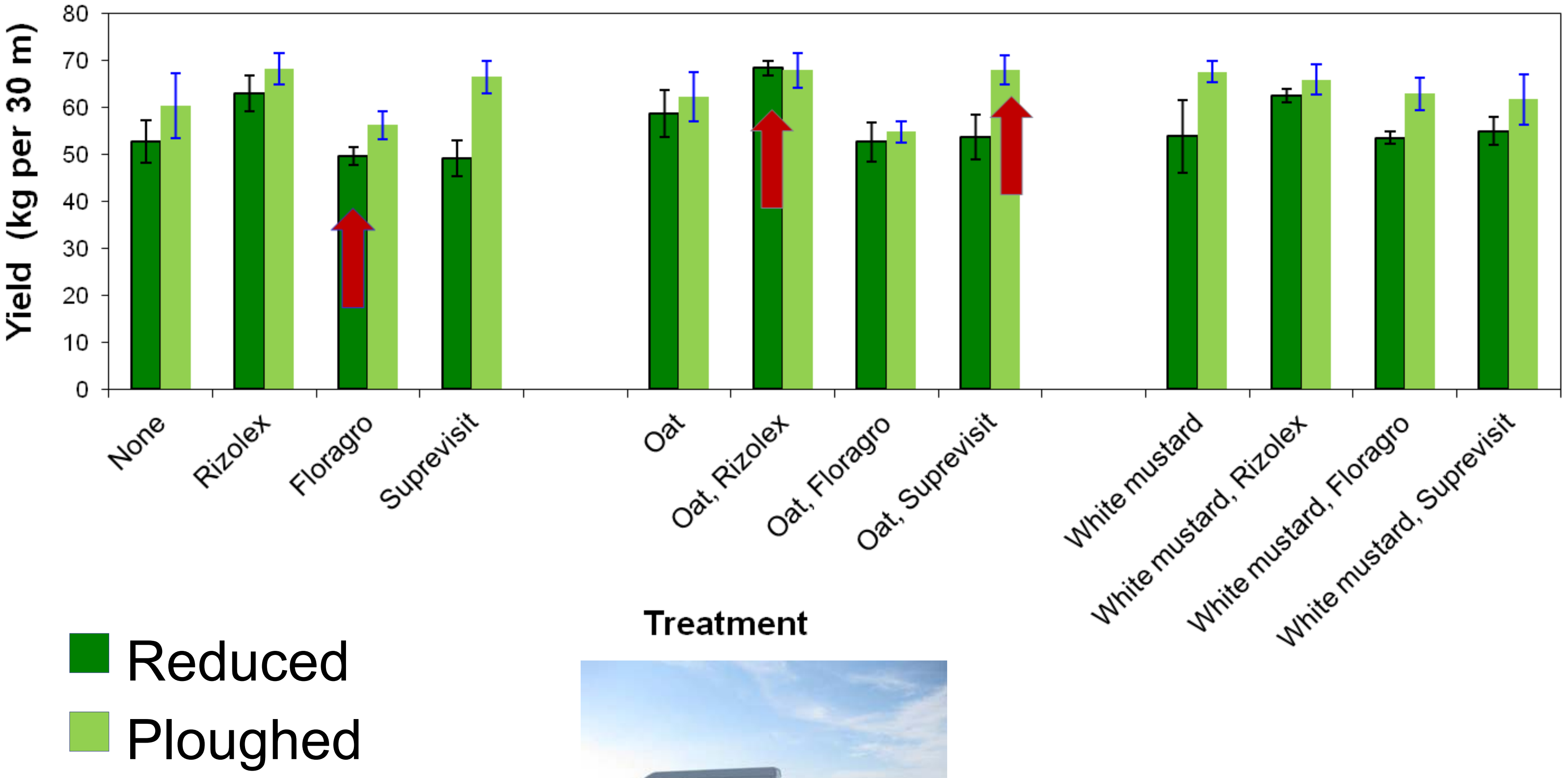
- Reduced soil treatment

  12 % higher yield

2009 High disease insidence

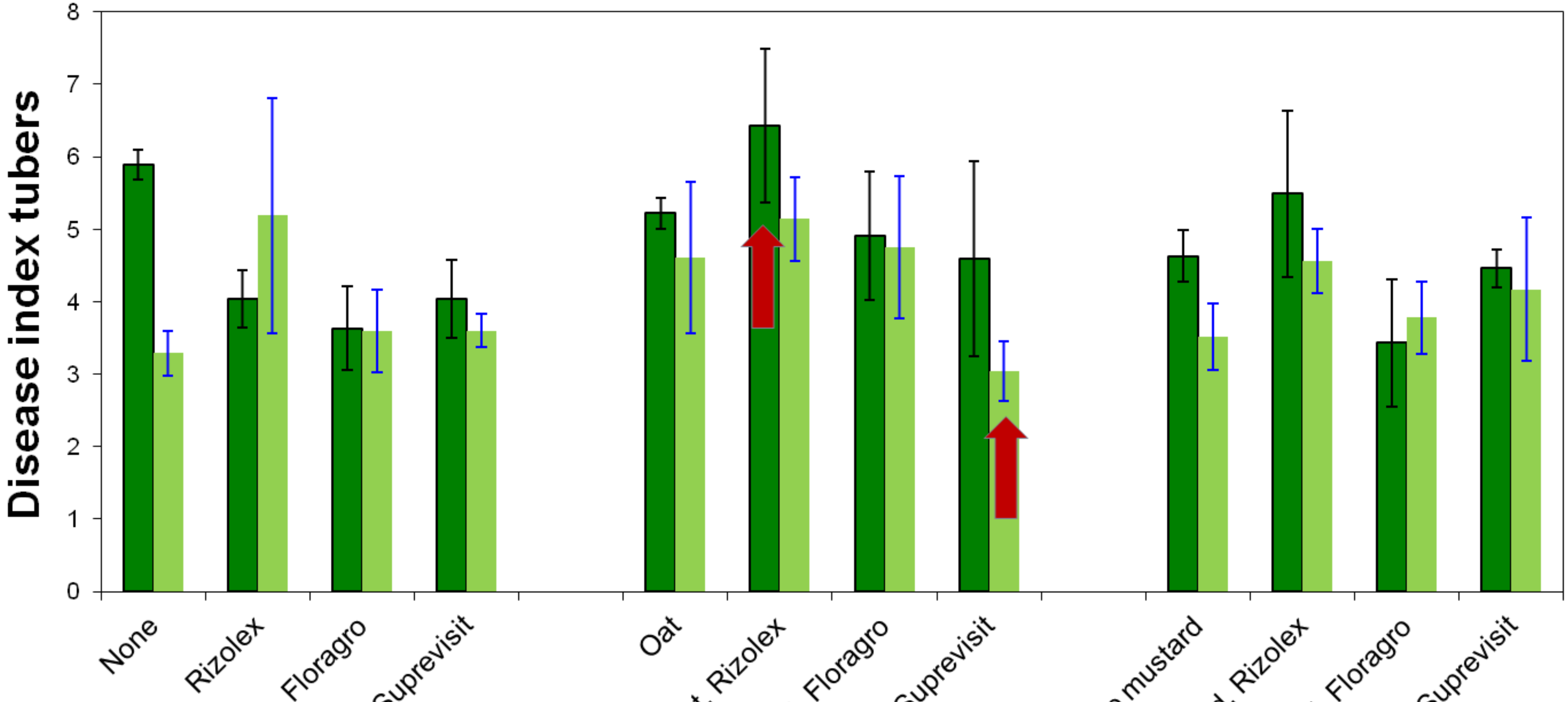
- Ploughing 15 % higher yield
- Rizolex 14 % higher yield

Results combined treatments 2009 high disease pressure



28 % difference

Results combined treatments 2009 high disease pressure



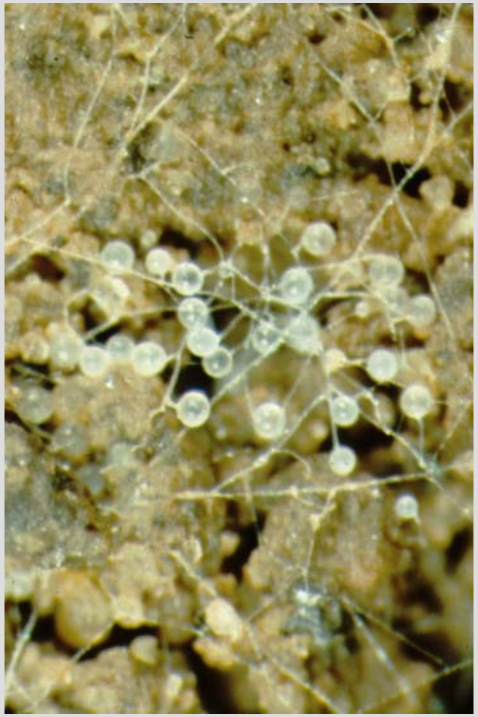
Difference 53%

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

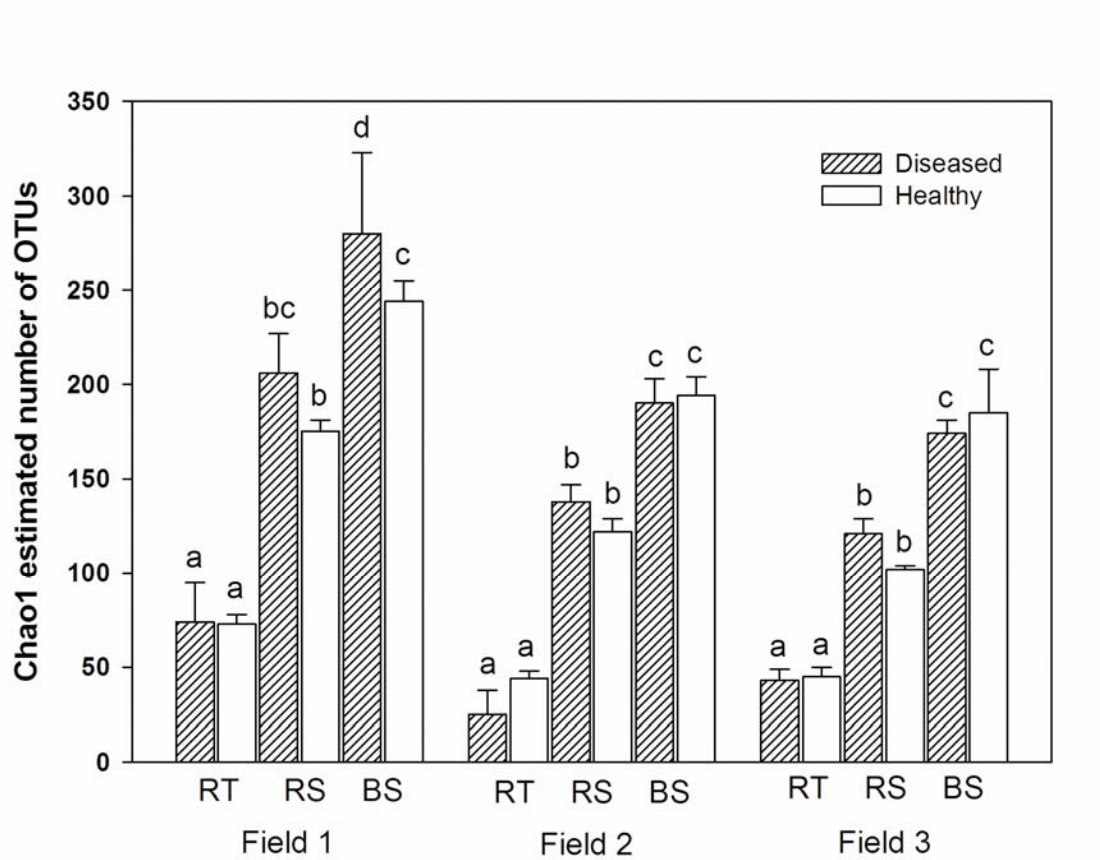
Beneficial interactions between plants and soil microbes



Outline

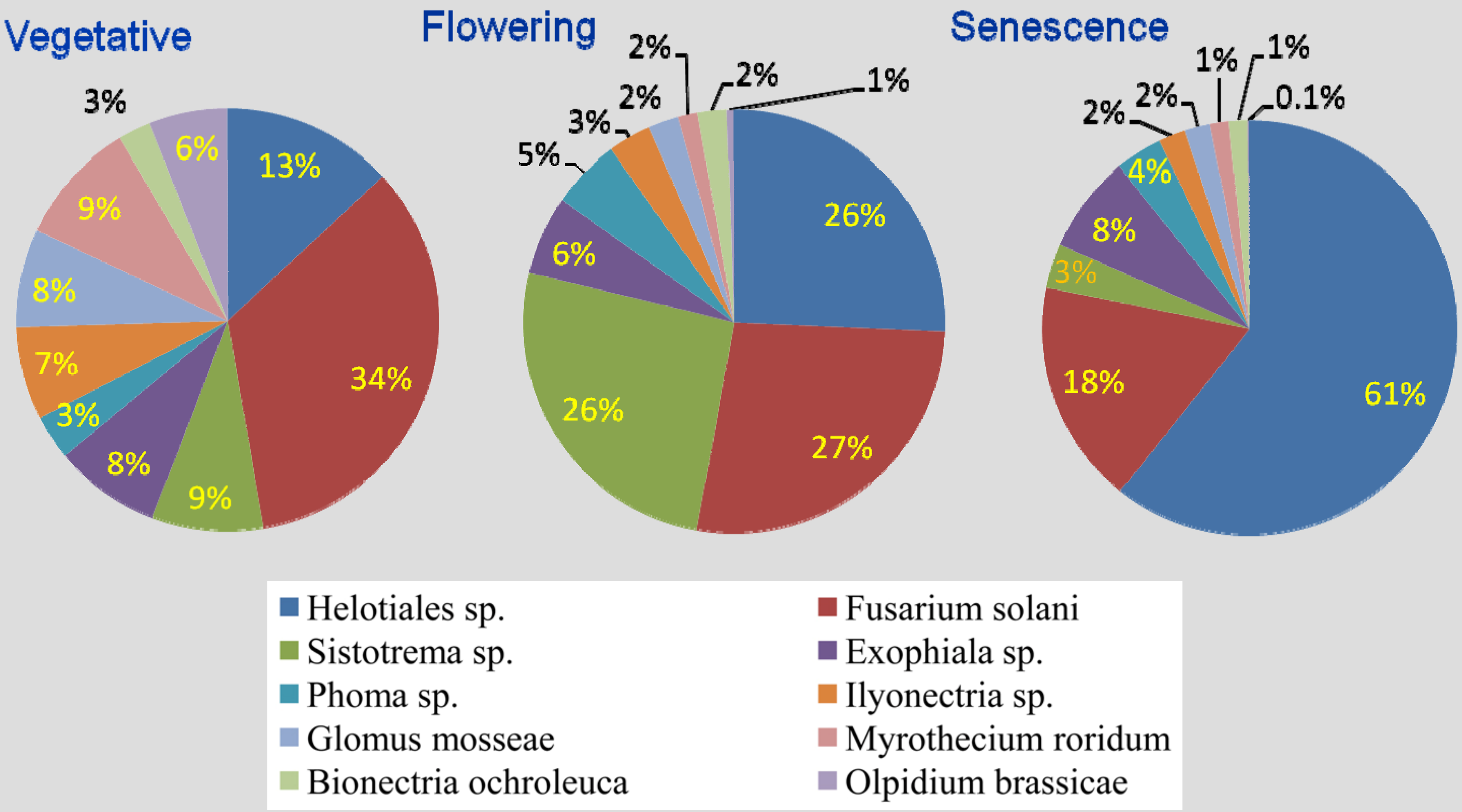
- Fungi in root and soil environments
- Arbuscular Mycorrhizal (AM) fungi – an example of a plant beneficial microorganism
- Interactions between AM and pathogenic oomycetes
- Conclusions
- (Mechanisms underlying increased AM plant tolerance against pathogens)

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)



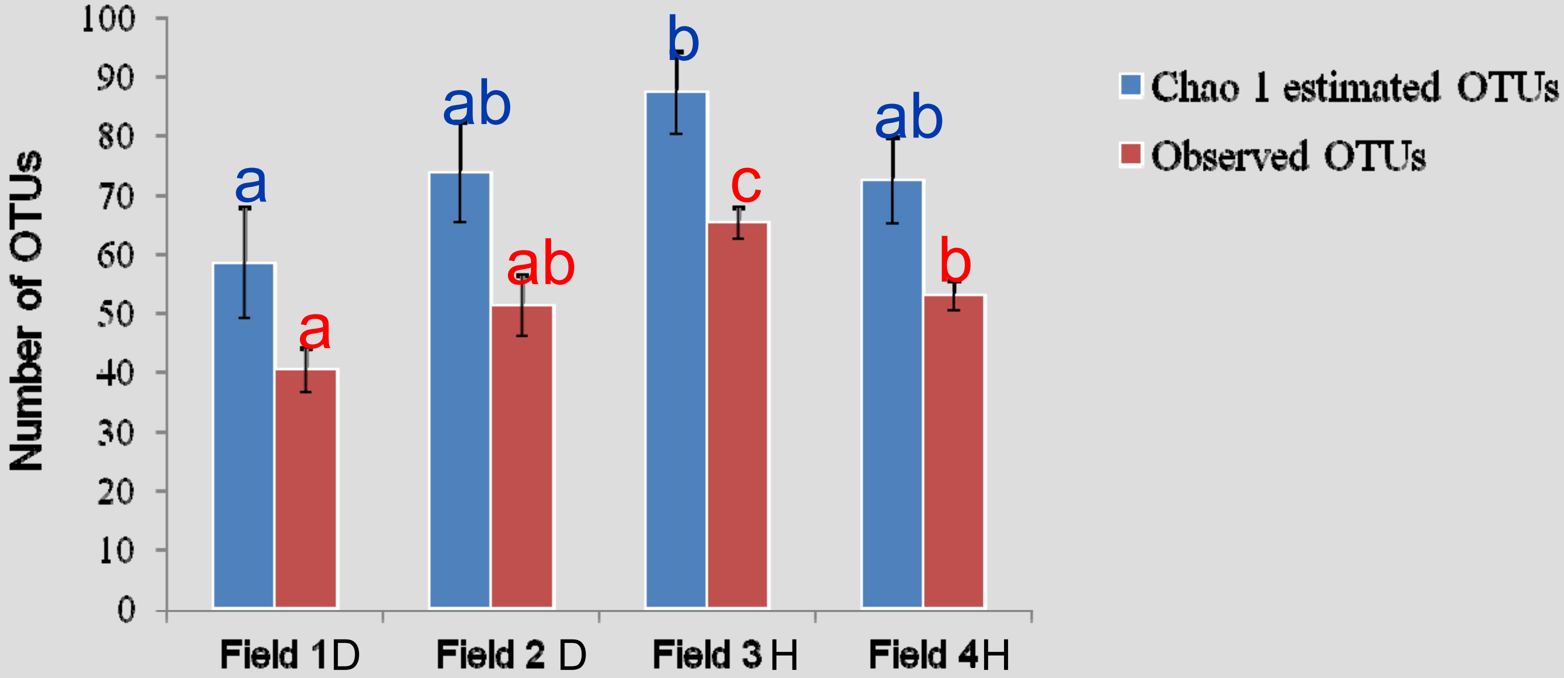
Yu *et al*. 2012, Plant and Soil 358:225-233

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 *P*-value
		Relative abundance of sequences (%)				
1	*Olpidium brassicae*	0.97 a	15.4 b	32.1 c	51.3 c	*** #
2	*Fusarium oxysporum*	29.5 a	31.9 a	24.7 a	11.2 b	**
3	***Archaeospora trappei***	**13.4 a**	**10.3 ab**	**6.9 ab**	**5.6 b**	**NS**
4	*Exophiala* 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	***Paraglomus* sp.**	**4.3 ab**	**5.6 a**	**3.8 ab**	**1.9 b**	*****
7	***Glomus mosseae***	**0.6 a**	**2.7 b**	**3.6 b**	**5.6 b**	**** #**
8	***G. caledonium***	**0.5 a**	**1.6 b**	**2.7 b**	**7.1 c**	***** #**
9	*Trichocladium asperum*	3.1 a	2.3 a	2.0 a	1.0 a	NS #
10	*F. solani*	1.6 a	0.6 a	0.8 a	2.3 a	NS #
11	*Eucasphaeria capensis*	1.9 a	1.2 a	1.1 a	0.3 b	NS #
12	*Xylariales* sp.	2.2 a	0.5 b	0.3 c	0.2 c	*** #
13	*Plectosphaerella cucumerina*	0.4 a	0.7 a	0.7 a	0.6 a	NS #
14	*Hypocreales* sp.	0.6 a	0.6 a	0.5 a	0.5 a	NS
15	*Arthrobotrys* sp.	0.8 a	0.3 b	0.3 b	0.5 ab	NS
19	*Phoma eupyrena*	0.15 ab	0.53 c	0.52 bc	0.08 a	* #
20	*Cryptococcus terreus*	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



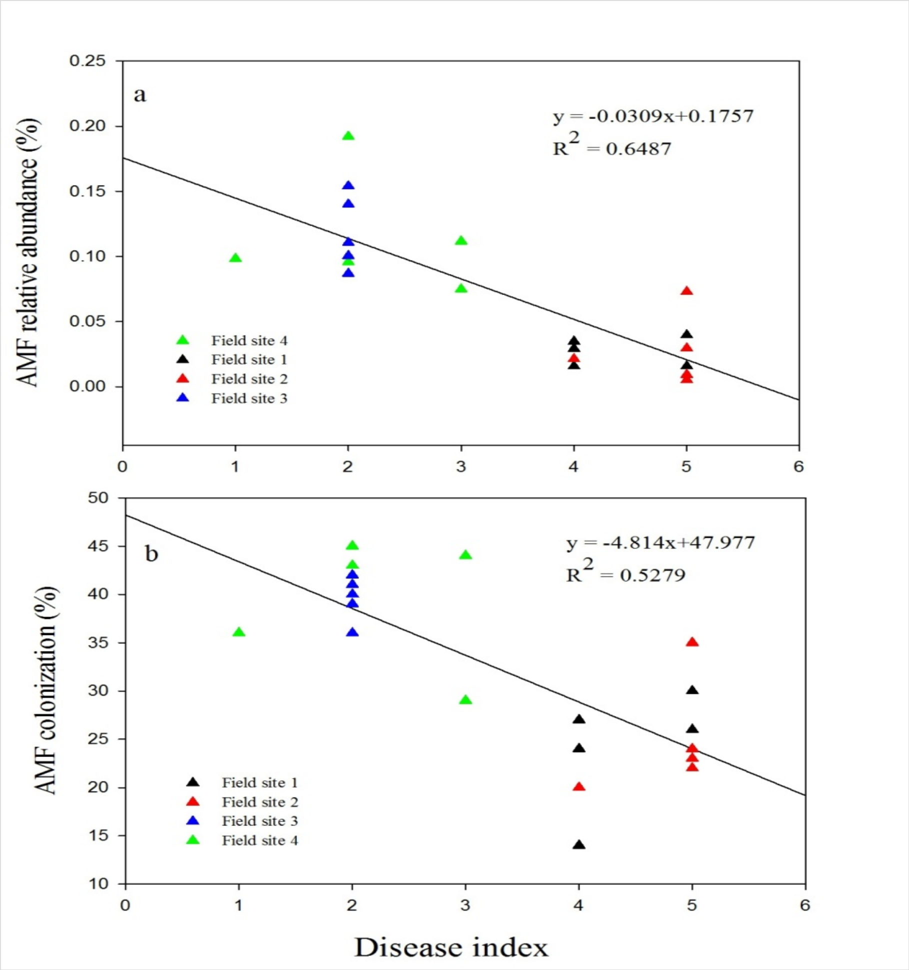
Yu *et al*. 2012, Plant and Soil 357:395-405

The15 most abundant of 142 fungal Operational Taxonomic Units (OTUs) in pea roots

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

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

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

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

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

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 -

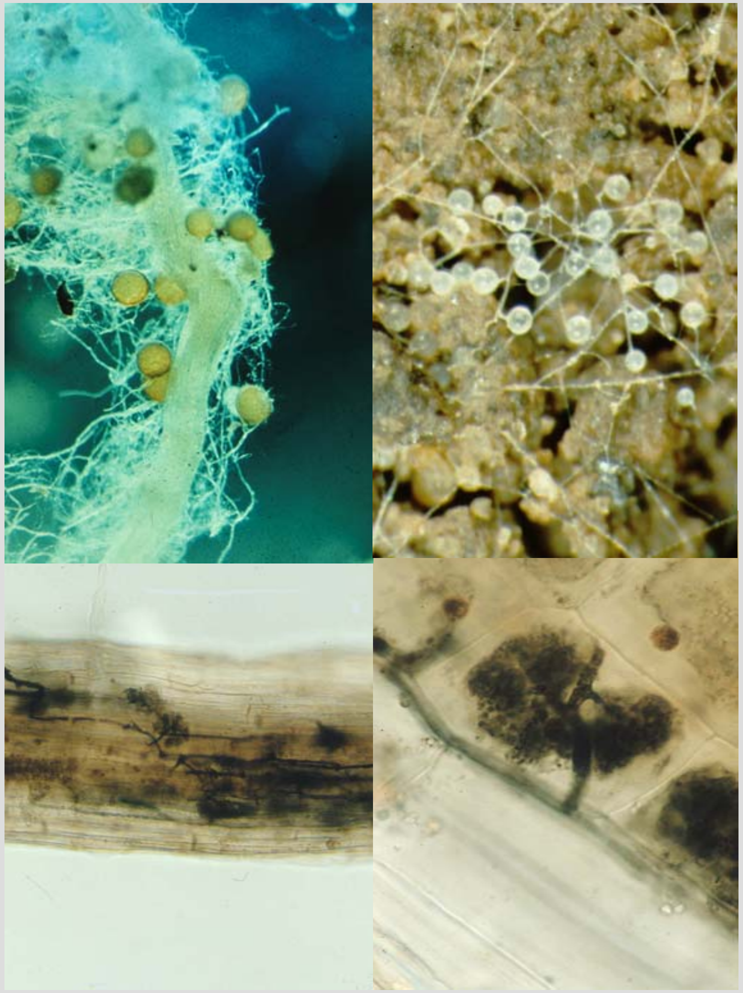
- nutrient uptake
- growth
- tolerance against abiotic stress
- tolerance against biotic stress

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

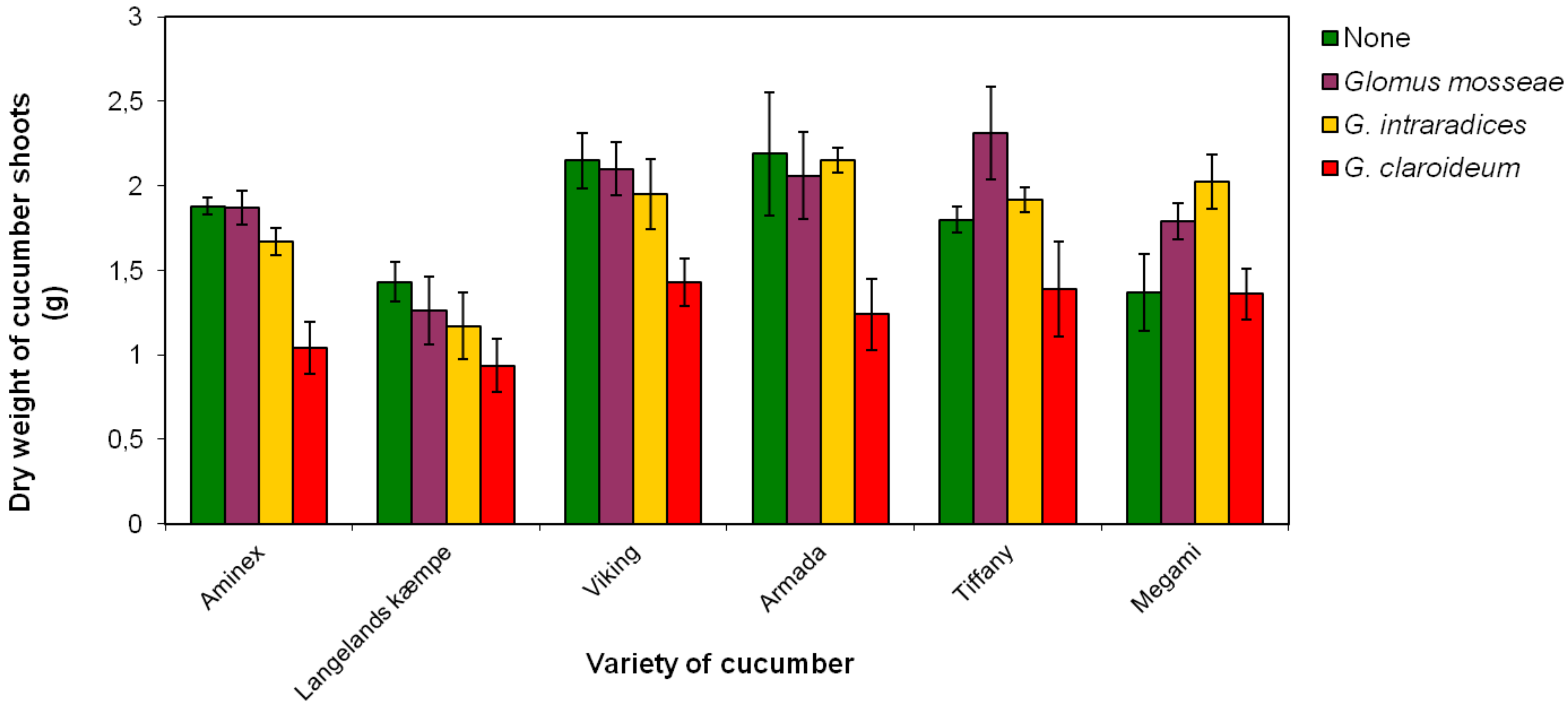
cover all of them

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

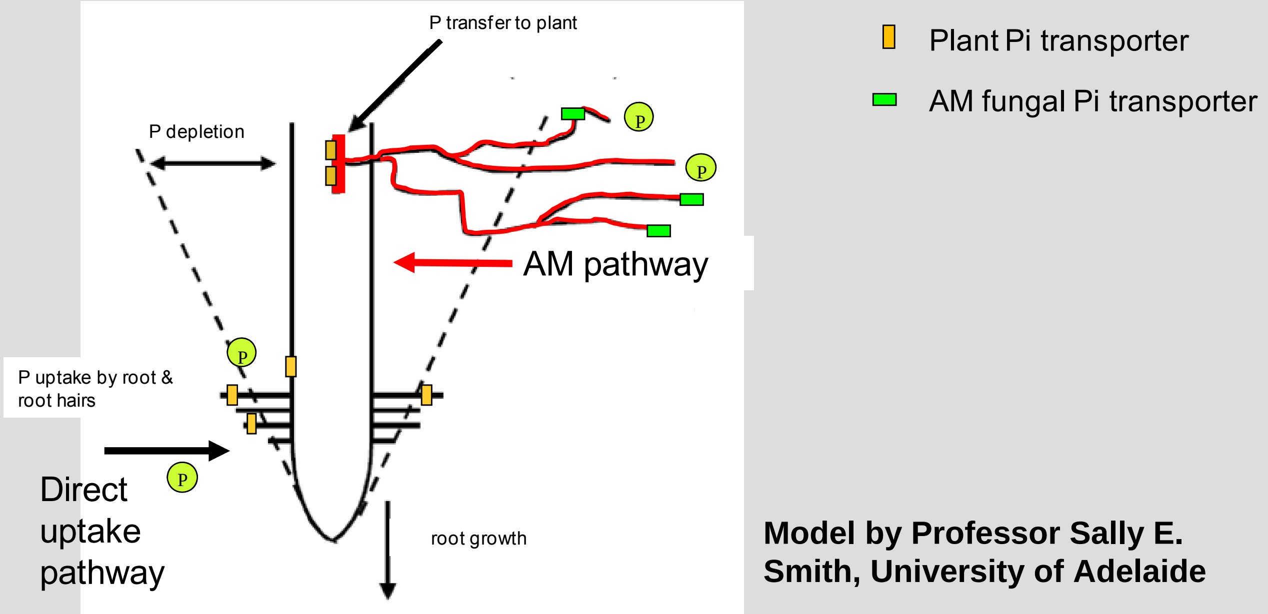


The role of AMF in plant growth



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



Model by Professor Sally E. Smith, University of Adelaide

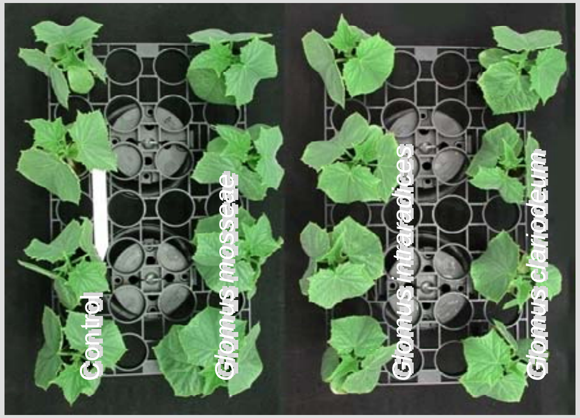
Nutrient concentrations in shoots of six varieties of cucumber inoculated with AM fungi

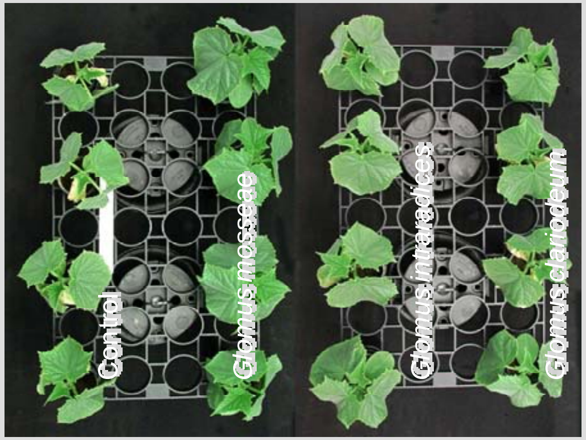
P values of a two-way analysis of variance * P≤0.05 ** P≤0.01 ***P≤0.001

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

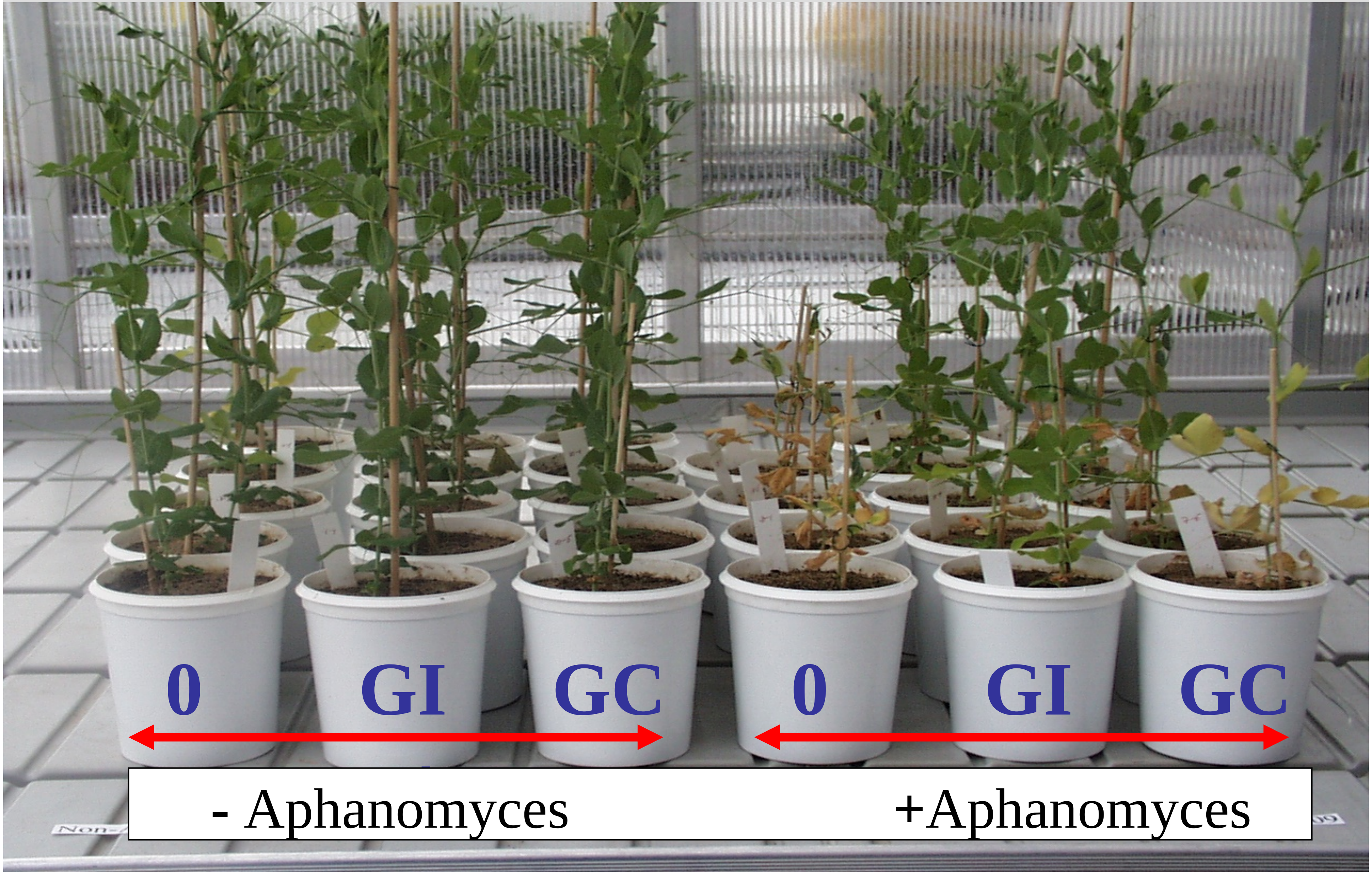




AM fungi as an example of a multifunctional plant beneficial microorganism - summary

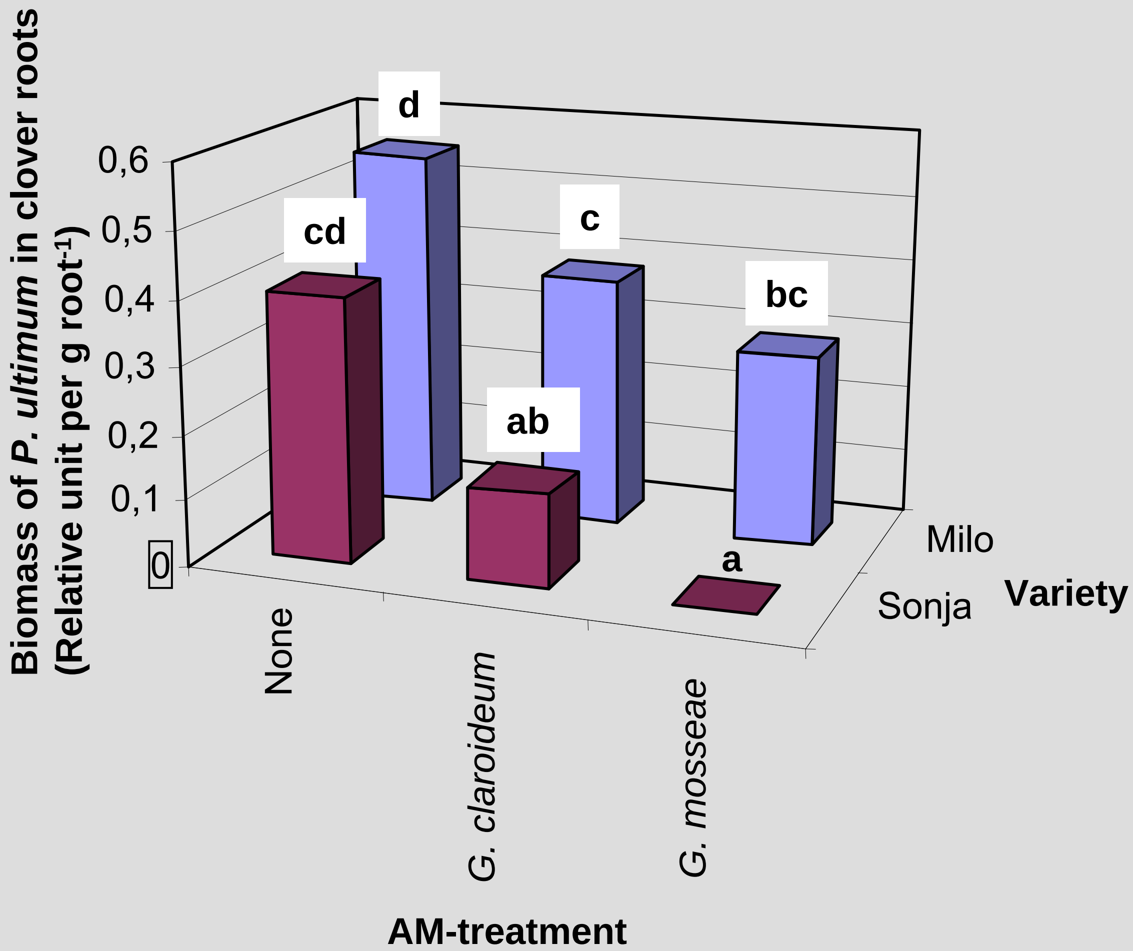
- **AM fungi**
  - Influence growth of plants
  - Influence uptake of nutrients
  - Increase plant stress tolerance
  - Indicate plant health?

Interactions between AM and pathogenic oomycetes



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

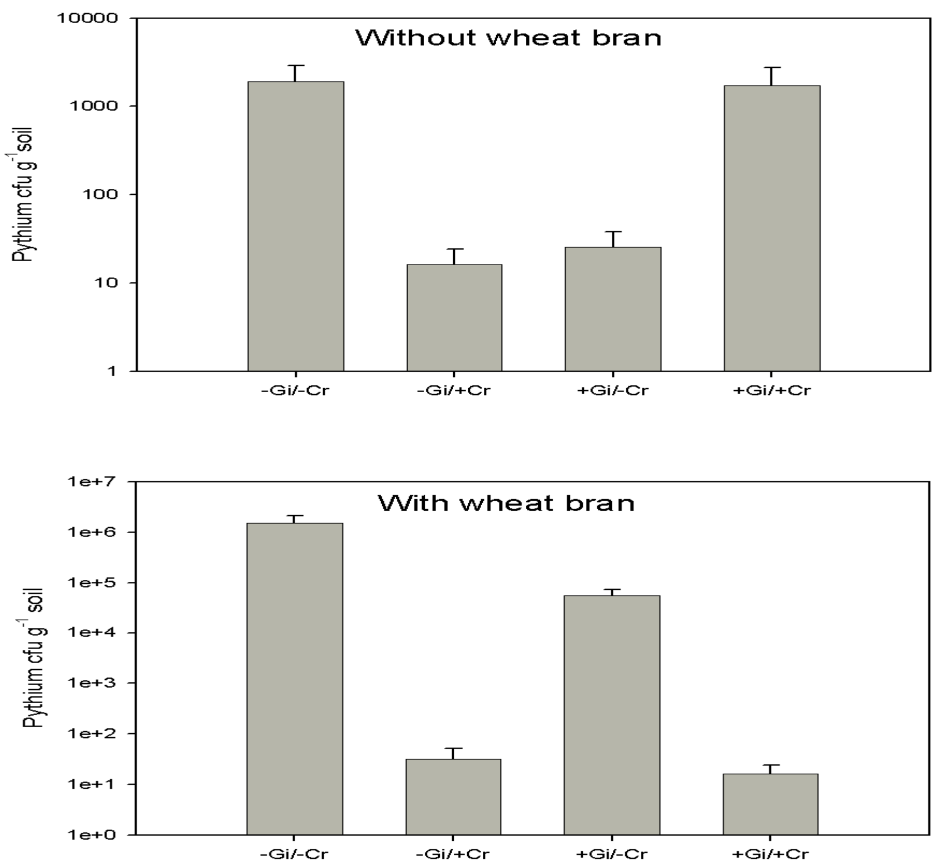
AM fungal effect on *Pythium ultimum* in white clover roots



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

Carlsen *et al.* 2008. Plant and Soil 302:33-43

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!!!