Agroecology for IPM III
Diseases

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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: *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>
<thead>
<tr>
<th>Antibiotic</th>
<th>Source</th>
<th>Target Pathogen</th>
<th>Disease</th>
<th>Reference</th>
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<tr>
<td>2, 4-diacetylphlorogluconol</td>
<td>Pseudomonas fluorescens F113</td>
<td><em>Pythium spp.</em></td>
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<td>Shanahan et al. (1992).</td>
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<td>Agrocin 84</td>
<td>Agrobacterium radiobacter</td>
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<td>Bacillomycin D</td>
<td>Bacillus subtilis AU195</td>
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<td>Lysobacter sp. strain SB-K88</td>
<td><em>Aphanomyces cochlioides</em></td>
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<td>Glotoxin</td>
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<td>Herbicolin</td>
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<td><em>Erwinia amylovora</em></td>
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<td>Pyrroloquinine, pseudane</td>
<td><em>Burkholderia cepacia</em></td>
<td><em>R. solani</em> and <em>P. pyricularia oryzae</em></td>
<td>Damping off and rice blast</td>
<td>Homma et al. (1989)</td>
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<td>Zwittermicin A</td>
<td><em>Bacillus cereus</em> UW85</td>
<td><em>Phytophthora medicaginis</em> and <em>P. aphanidermatum</em></td>
<td>Damping off</td>
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Mycoparasitism of *Rhizoctonia* by *Trichoderma*
Nematode trapping fungi
Fungal grazing by Collembola

Foto: John Larsen
<table>
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<tr>
<th>Bacterial strain</th>
<th>Plant species</th>
<th>Bacterial determinant</th>
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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*

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*
Biological management of plant diseases – a summary

- There are several modes of actions in biological management of plant diseases, and this is important to 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
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
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)
• \textit{R. solani} infection in tubers
• Nematodes in soil from selected plot
• \textit{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

Yield (kg per 30 m)

- None
- Rizolex
- Floragro
- Suprevisit
- Oat
- Oat, Rizolex
- Oat, Floragro
- Oat, Suprevisit
- White mustard
- White mustard, Rizolex
- White mustard, Floragro
- White mustard, Suprevisit

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

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

<table>
<thead>
<tr>
<th>OTU No.</th>
<th>Best hit in GenBank</th>
<th>No OF</th>
<th>1OF</th>
<th>2OF</th>
<th>3OF</th>
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<td>Olpidium brassicae</td>
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<td>32.1 c</td>
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<td>31.9 a</td>
<td>24.7 a</td>
<td>11.2 b</td>
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<td>Archaeospora trappei</td>
<td>13.4 a</td>
<td>10.3 ab</td>
<td>6.9 ab</td>
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<td>Exophiala sp.</td>
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<td>5.8 b</td>
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<td>2.7 b</td>
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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

<table>
<thead>
<tr>
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<td>1 (D) 2 (D) 3 (H) 4 (H)</td>
<td>Relative abundance of sequences (%)</td>
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<td><em>Plectosphaerella cucumerina</em></td>
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<td><em>Microdochium bolleyi</em></td>
<td>0.5 a 2.3 b 0.6 a 0.4 a</td>
<td>**</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Uncultured fungus</td>
<td>2.3 a 0.3 a 0.2 a 0.4 a</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

*Yu et al. 2012, Plant and Soil 357:395-405*
Abundance of AM fungi in *Pisum sativum* roots correlates with root health status

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

26 different OTUs of AM fungi in these roots
Fungal pea root health indicators as calculated using Indicator Species Analysis (ISA)

<table>
<thead>
<tr>
<th>OTU ID</th>
<th>Indicator value</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Glomus mosseae</em></td>
<td>97.9</td>
</tr>
<tr>
<td><em>G. caledonium</em></td>
<td>90.3</td>
</tr>
<tr>
<td><em>Mortierella elongata</em></td>
<td>84.4</td>
</tr>
<tr>
<td>No ID</td>
<td>79.5</td>
</tr>
<tr>
<td><em>Exophiala salmonis</em></td>
<td>75.5</td>
</tr>
<tr>
<td><em>Cladosporium cucumerinum</em></td>
<td>72.4</td>
</tr>
<tr>
<td><em>G. versiforme</em></td>
<td>64.7</td>
</tr>
<tr>
<td><em>G. mosseae</em></td>
<td>64.5</td>
</tr>
<tr>
<td><em>M. elongata</em></td>
<td>50.1</td>
</tr>
</tbody>
</table>

• 123 OTU’s were identified in these roots
• These nine showed significant health indicator values (P≤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

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

<table>
<thead>
<tr>
<th></th>
<th>Tot. N</th>
<th>P</th>
<th>K</th>
<th>Mg</th>
<th>Ca</th>
<th>Na</th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Cu</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cucumber ssp. (V)</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>0.21</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>AM fungal species(F)</td>
<td>0.11</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>0.09</td>
<td>**</td>
<td>***</td>
<td>***</td>
<td>*</td>
</tr>
<tr>
<td>Interaction VxF</td>
<td>*</td>
<td>*</td>
<td>*</td>
<td>0.36</td>
<td>**</td>
<td>***</td>
<td>0.14</td>
<td>0.41</td>
<td>0.15</td>
<td>*</td>
</tr>
</tbody>
</table>

Model by Professor Sally E. Smith, University of Adelaide
The role of AMF in alleviation of biotic stress

<table>
<thead>
<tr>
<th>AM fungus</th>
<th><em>Pythium ultimum</em></th>
<th>Dry weight of 28-day old shoots (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>1.63 d</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.15 a</td>
</tr>
<tr>
<td><em>Glomus mosseae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.64 d</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.56 cd</td>
</tr>
<tr>
<td><em>G. intraradices</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.44 bc</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.31 b</td>
</tr>
<tr>
<td><em>G. claroideum</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>1.35 b</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>1.17 a</td>
</tr>
</tbody>
</table>
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

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

![Bar chart showing the effects of AM fungi and *Clonostachys rosea* on *Pythium* in soil with and without wheat bran.](chart.png)
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!!!