# Can strawberry crown rot caused by *Phytophthora cactorum* be controlled by using beneficial micro-organisms?

*Mauritz Vestberg, Päivi Parikka, Saila Karhu, Riitta Kemppainen, Sanna Kukkonen and Risto Tahvonen*

MTT Agrifood Research Finland, Horticultural Research, Laukaa, Finland

11.4.2014

# Background

- Strawberry crown rot (*Phytophthora cactorum*) was first detected in 1952
- The disease is now found in most European countries, USA and in some parts of Asia and Africa.
- After the world-wide banning of soil fumigation with methyl bromide, the control of strawbery crown rot has to rely on other methods. This has opened new possibilites for the use of biocontrol agents and growth stimulants.

Photo: Päivi Parikka

# Background, endophytic *Pseudomonas*

- Earlier *in vitro* studies at MTT/Piikkiö have shown that endophytic *Pseudomonas infrequens and P. aeruginosa* isolated from strawberry had the potential to decrese the damage causeed by crown rot and grey mold in strawberry.

- A field experiment (artificial *Phytophthora* inoculation) with endophytic *P. fluorescens* showed slower development of crown rot symptoms. However, at the end of the growing season, the level of crown rot was similar in endophyte inoculated as in non-inoculated

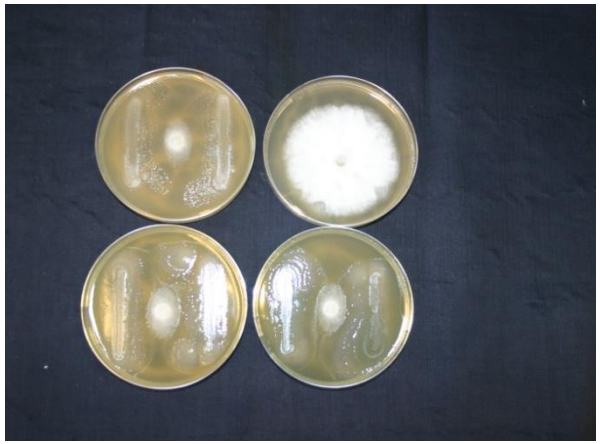
# Background, Arbuscular Mycorrhizal fungi (AMF)

- AMF have been found to reduce disease symptoms caused by fungal pathogens such as *Phytophthora, Gaeumannomyces, Fusarium, Chalara (Thielaviopsis), Pythium, Rhizoctonia, Sclerotium, Verticillium, Aphanomyces,* and for nematodes such as *Rotylenchus, Pratylenchus* and *Meloidogyne.*

- AMF strains isolated from Finland have been found to increse growth and yield of strawberry. A collection of living cultures of AMF is maintained at MTT/Laukaa

# Objective

- To test the ability of AMF strains and endophytic bacteria of the *Pseudomonas*, both isolated from Finland, to control strawberry crown rot (*Phytophthora cactorum*) in greenhouse tests.
- Micro-organisms have been tested separately and in mixtures
- **Sub-Task 2.3.2. of EUBerry. Improved disease management by biocontrol and other sustainable methods**

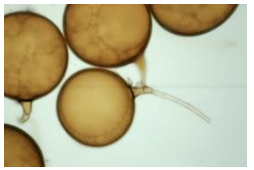


*P. aeruginosa/P. cactorum*

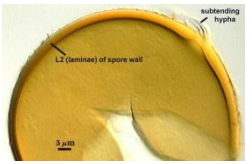
AMF

*Claroideoglomus claroideum*

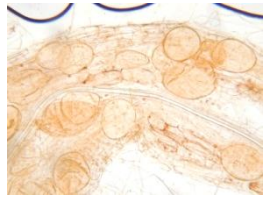
*Glomus hoi*



*Funneliformis mosseae*




*Claroideoglomus etunicatum*



*Rhizophagus intraradices*

# Materials and methods

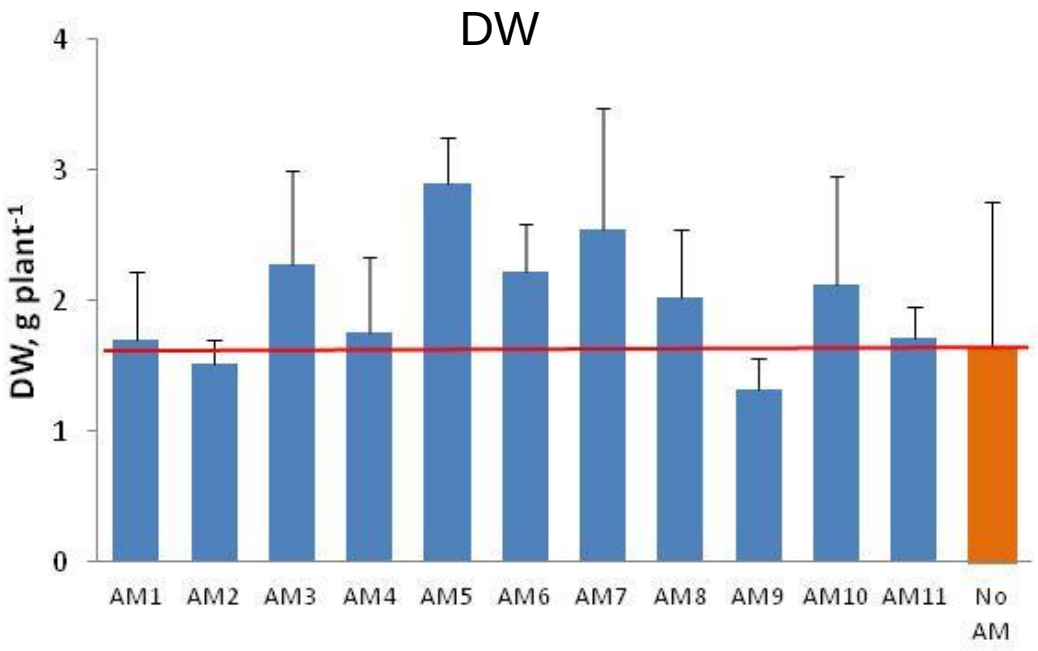
- Greenhouse experiments were carried out on strawberry artificially inoculated with zoosporangia suspensions of *Phytophthora cactorum.*
- Separate experiments with AMF and endophytic bacteria were first carried out. Therafter their mixtures were tested. In an on-going experiment also triple combinations AMF/*Pseudomonas/*disease suppressive compost will be tested.
- Two *Pseudomonas* species, several AMF strains and a commercial biocontrol product (Prestop, *Gliocladium catenulatum,* Verdera Oy, Finland*),* but no chemical fungicide, have been tested
- Experiments have been carried out in micropropagated strawberry ’Jonsok’, but also in using runner plants of ’Jonsok’.

# Parameters estimated

- **Shoot vigor (health)** were recorded weekly and at harvest on a rating scale 0-5 (0 = wilted, 5 = with no symptoms)
- **Shoot fresh and dry weight** at harvest
- The number of fully developed leaves, developing leaves, **wilted leaves** and shoot height were recorded weekly and at harvest
- **Crown discoloration** from *Phytophthora* inoculated pots at harvest on a rating scale 0-3 (0 = no discoloration, 3 = strongly brown). In expt 2/2012, 0-1 (0 = no discoloration, 1 = strongly brown
- **Root health** 1-3 (1=severy damaged, 3=healthy)
- **AMF root colonization** of AMF inoculated plants

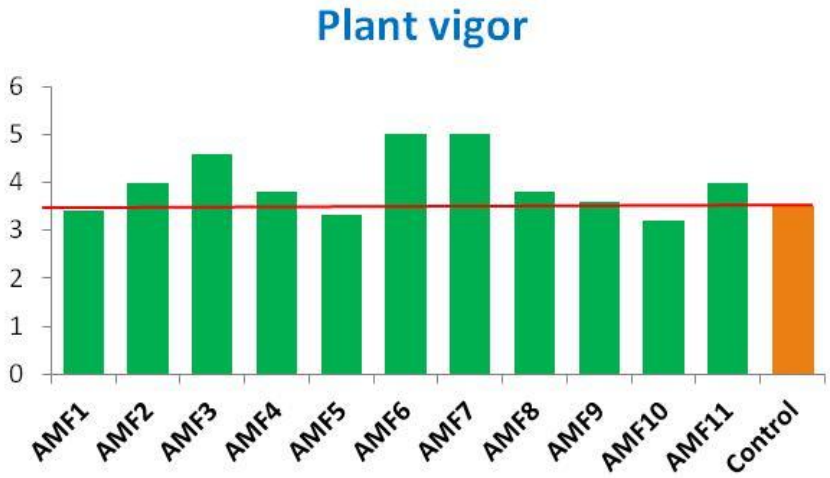
# Results 2011 – screening of 12 AMF strains

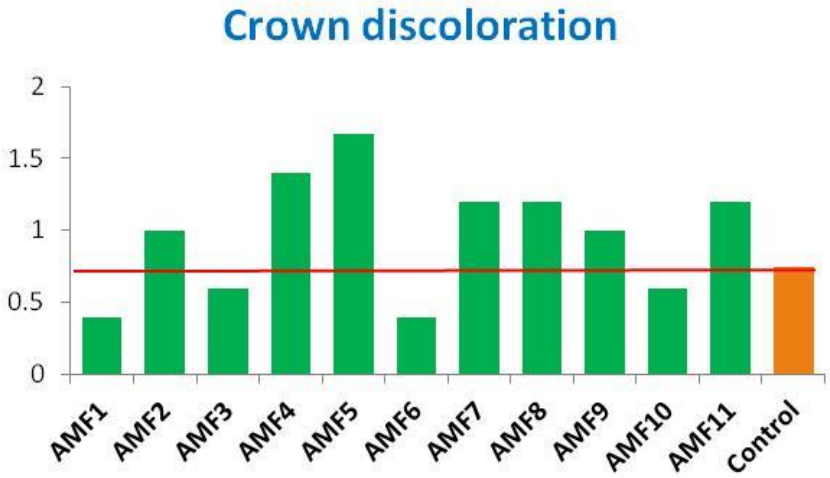
Experiment in rockwool – moderate development of disease




- The mycorrhizal strains AM3, AM5 and AM10 seemed to improve strawberry growth

# Results 2011 – screening of 12 AMF strains




AMF strains 3, 6 and 7 slightly improved plant vigor . No strain had negative effect on plant vigor!

Strains 1 and 6 reduced crown rot symptoms. AMF- strains 4, 5, 7, 8 and 11 had a negative impact- more discoloration than in control (orange bar).

# Results 2011 – screening of 12 AMF strains

## Summary

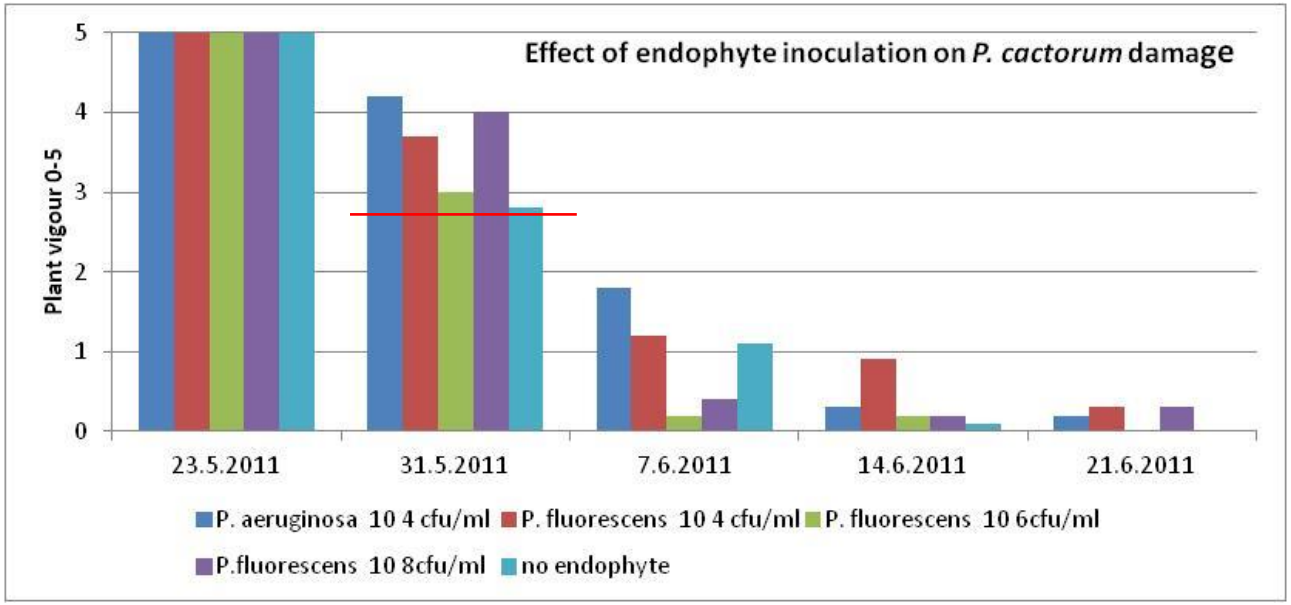
Three AMF strains were chosen for further experiments:

- (1) *Claroideoglomus claroideum* BEG31
- (3) *Glomus hoi* V95
- (6) *Glomus hoi* V307

| AMF strain | Shoot vigor 0-5 | Dead leaves No | Crown discoloration 0-3 |
|---|---|---|---|
| 1 | 0 | + | + |
| 2 | 0 | + | 0 |
| 3 | + | + | 0 |
| 4 | 0 | + | - |
| 5 | 0 | 0 | - |
| 6 | + | + | + |
| 7 | + | 0 | - |
| 8 | 0 | 0 | |
| 9 | 0 | 0 | 0 |
| 10 | 0 | - | 0 |
| 11 | 0 | + | - |

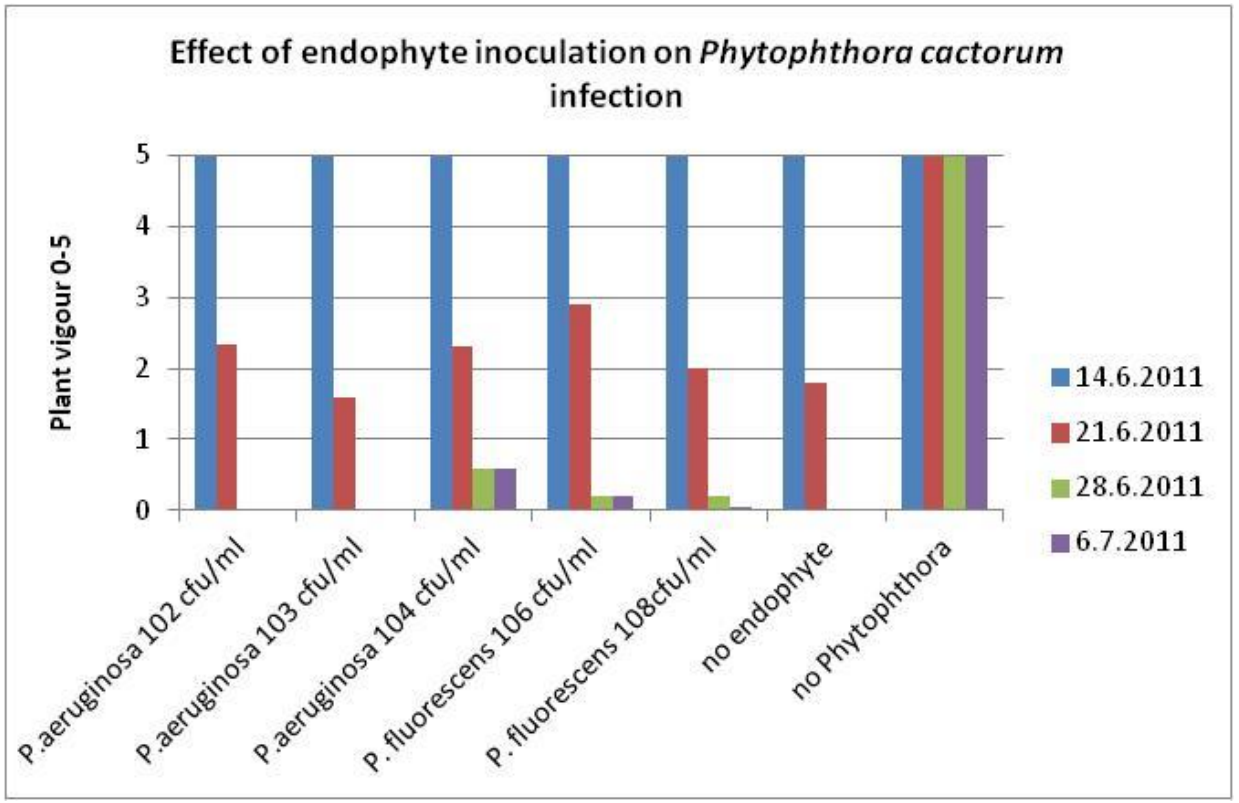
# Results 2011 – endophytic *Pseudomonas*, microplants

Experiment in rockwool – strong development of disease




- Indications of a higher plant vigor due to bacterial endophytes were observed on 31 May, 2011. Thereafter the disease development was too strong to allow comparisons between treatments.

# Results 2011 – endophytic bacteria, runner plants

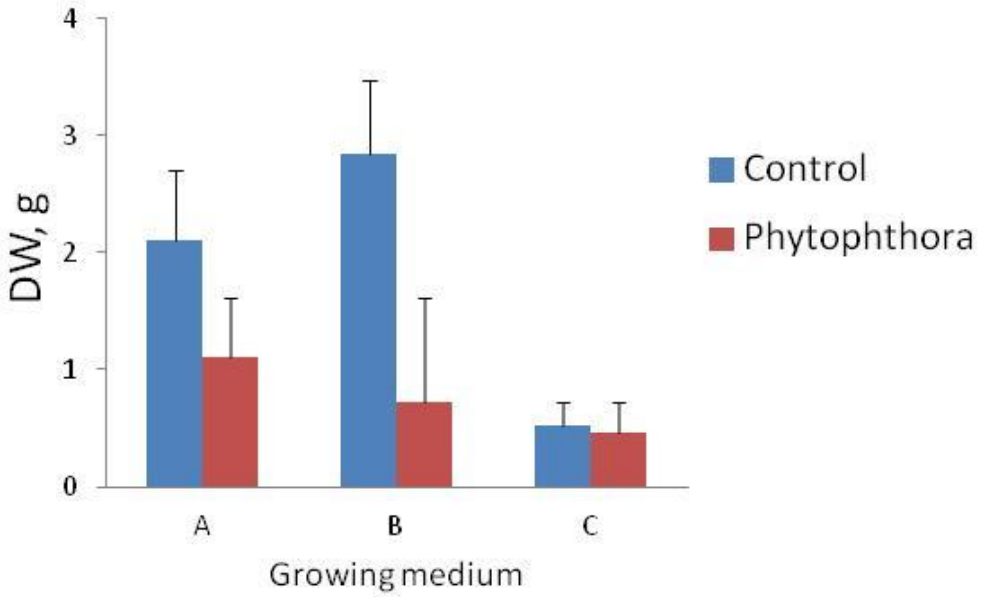



-In rockwool *P. cactorum* infection was very strong
-No inoculation could protect plants against *P. cactorum*
-The experiments will be continued in organic + sand substrate

# Results 2012 – AMF, Impact of growing medium on *Phytophthora* severity and AMF impact

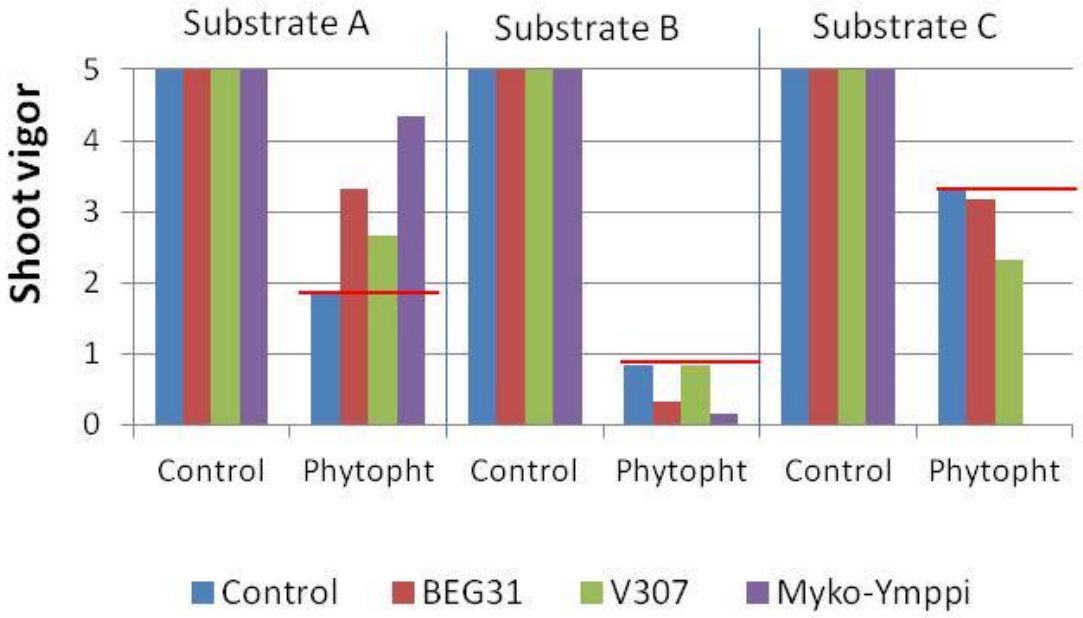
Growing media

A. Sand (75%) + kaolin (10%) + Perlite (10% + peat (5%)
B. Sand (40%) + Perlite (30%) + clay (10%) + peat (20%)
C. Sand (50% + clay (50%)




*Phytopthora* inoculation significantly decreased strawberry DW in growing media A and B, but not in C

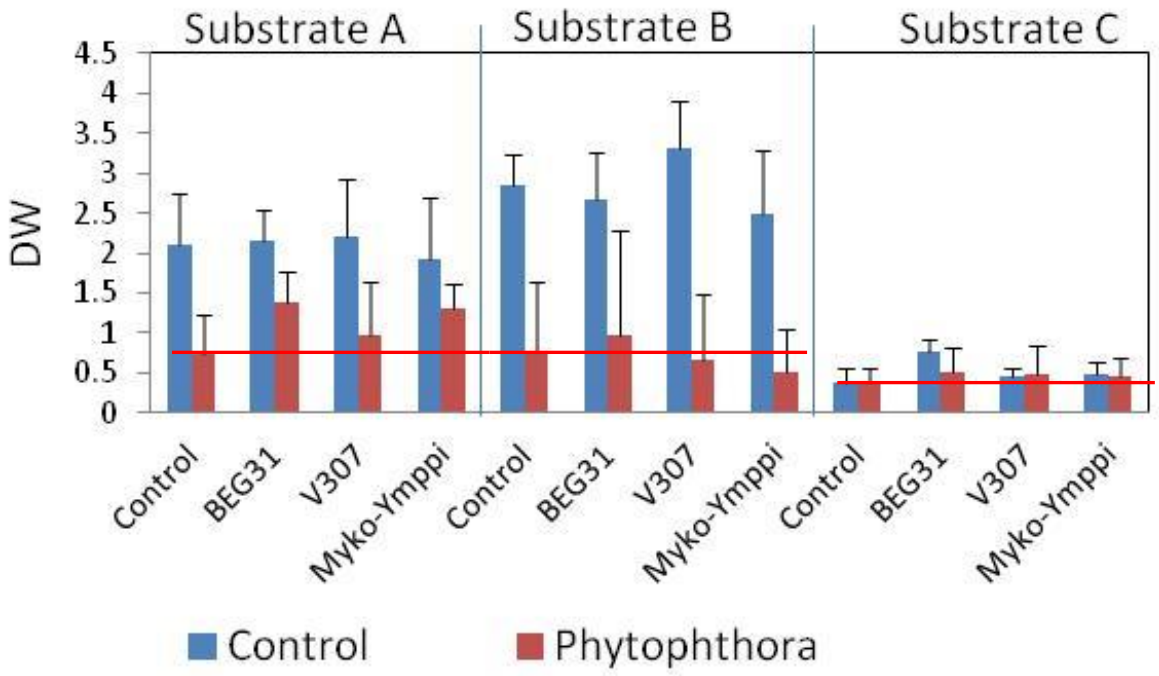
# Results 2012 – Impact of growing medium on *Phytophthora* severity and AMF impact. Continued




AMF inoculation slightly improved shoot vigor in strawberry challenged to *P. cactorum*, but only in substrate A.

# Results 2012 – Impact of growing medium on *Phytophthora* severity and AMF impact. Continued

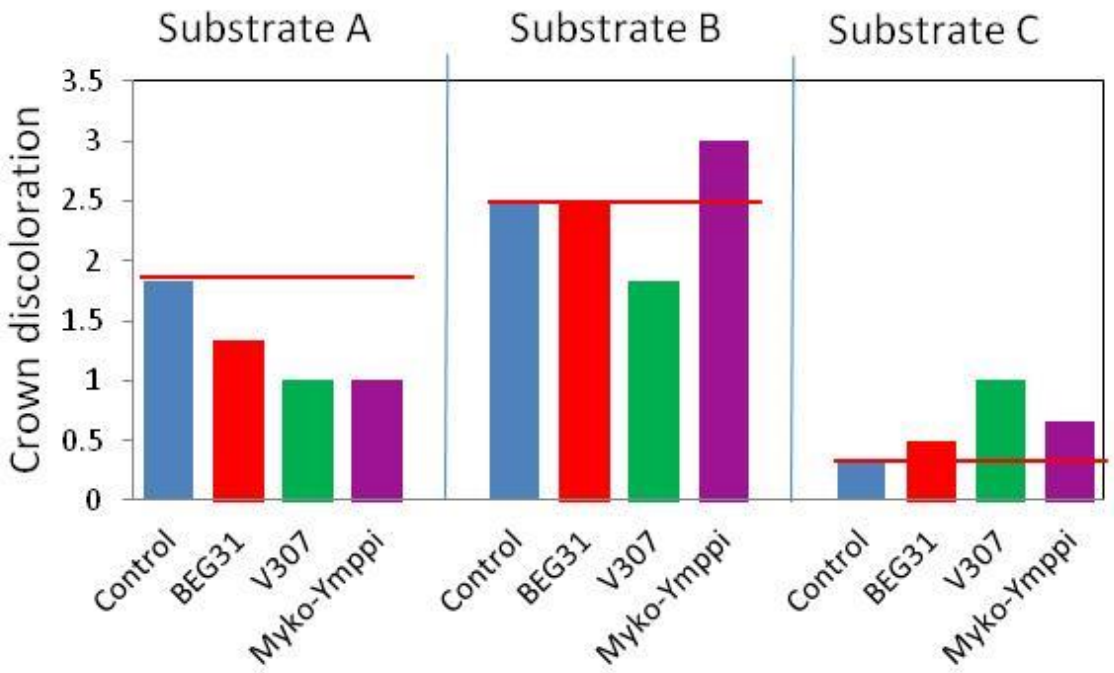
**Shoot DW**




Compared with the control without *Phytopthora,* AMF did not significantly improve control of crown rot measured as shoot DW

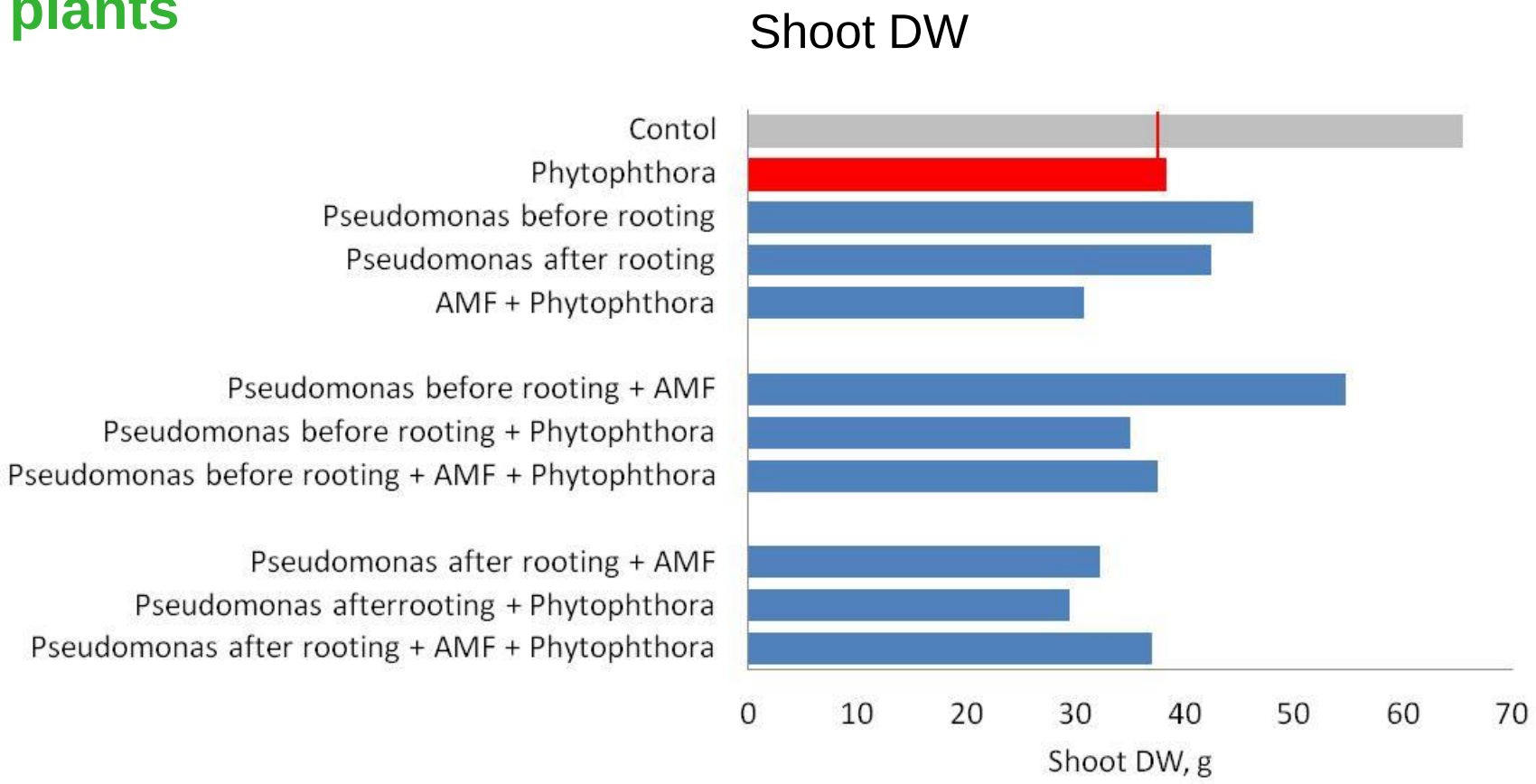
# Results 2012 – Impact of growing medium on *Phytophthora* severity and AMF impact. Continued

**Crown discoloration**




AMF (V307 and Myko-Ymppi) decreased crown discoloration symptoms in substrate A.

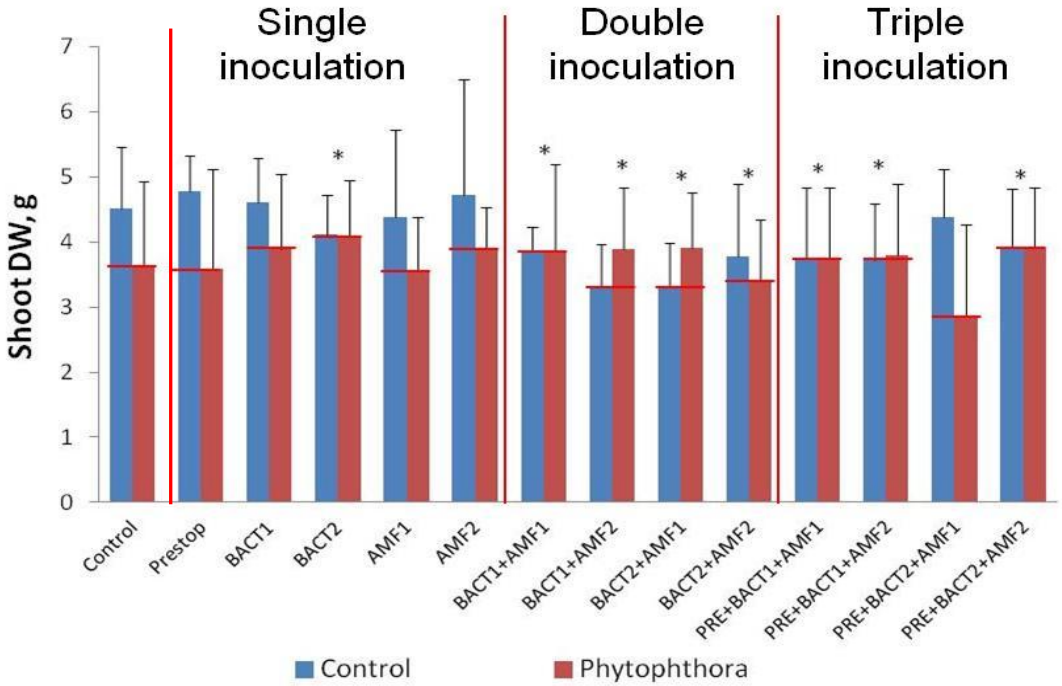
# Results 2012. *Pseudomonas* (and AMF), runner plants




- *Pseudomonas* inoculation dectreased shoot growth
- *Pseudomonas* inoculation, irrespective of before or after rooting, did not control *Phytophthora*

# Results 2012 – AMF and *Pseudomonas*

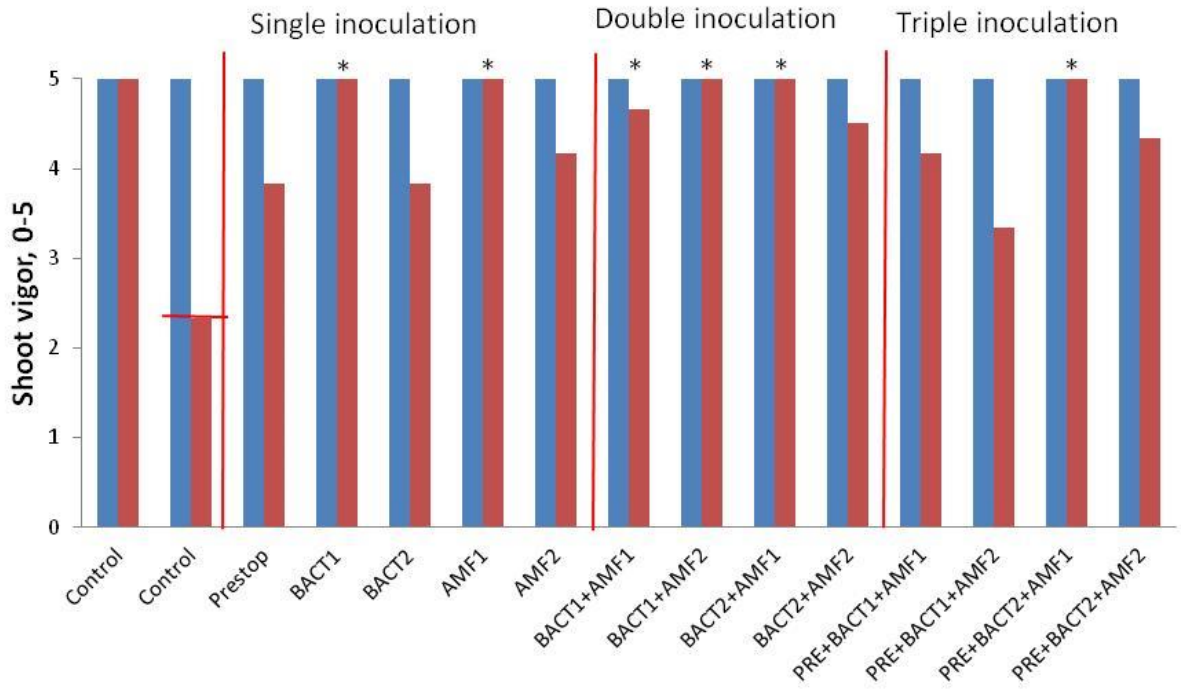
Shoot DW




- Small decrease in shoot DW due to *Phytophthora* inoculation
- The biological product Prestop did not affect shoot DW
- Of single inoculants, only *P. aeruginosa* (BACT2) affected shoot DW
- **All but one of double and tripe inoculations affected shoot DW positively**

# Results 2012 – AMF and *Pseudomonas*

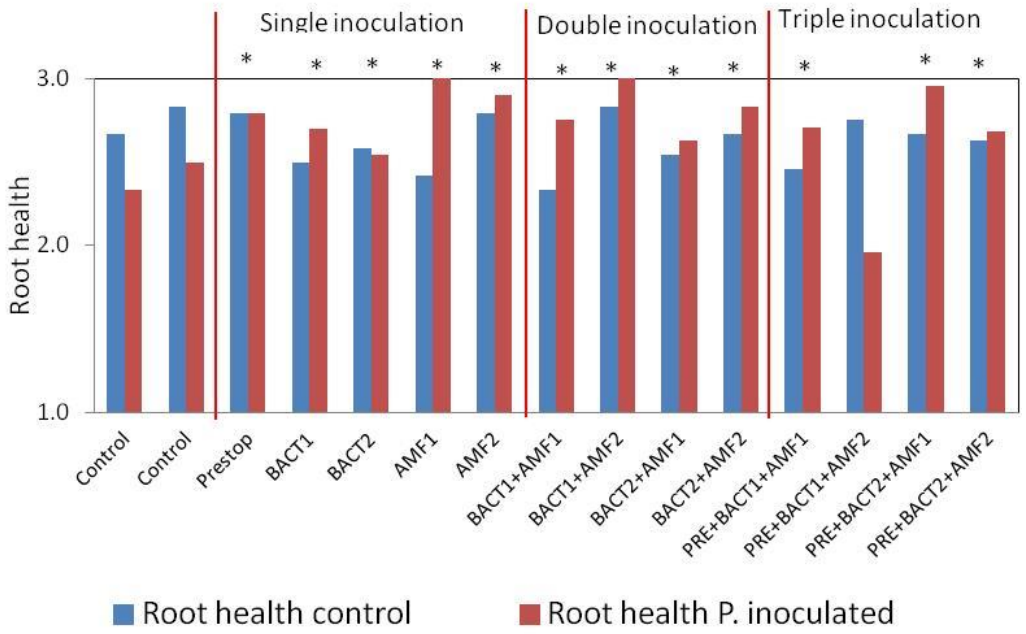
Shoot vigor




- Shoot vigor in the control was about 50% of not *Phytophthora* inoculated
- Of single inoculants, in particular *P. fluorescens* (BACT1) and AMF1 affected shoot vigor positively.
- **Double inoculations seemed to affect shoot vigor more positively than single and triple inoculations**

# Results 2012 – AMF and *Pseudomonas*

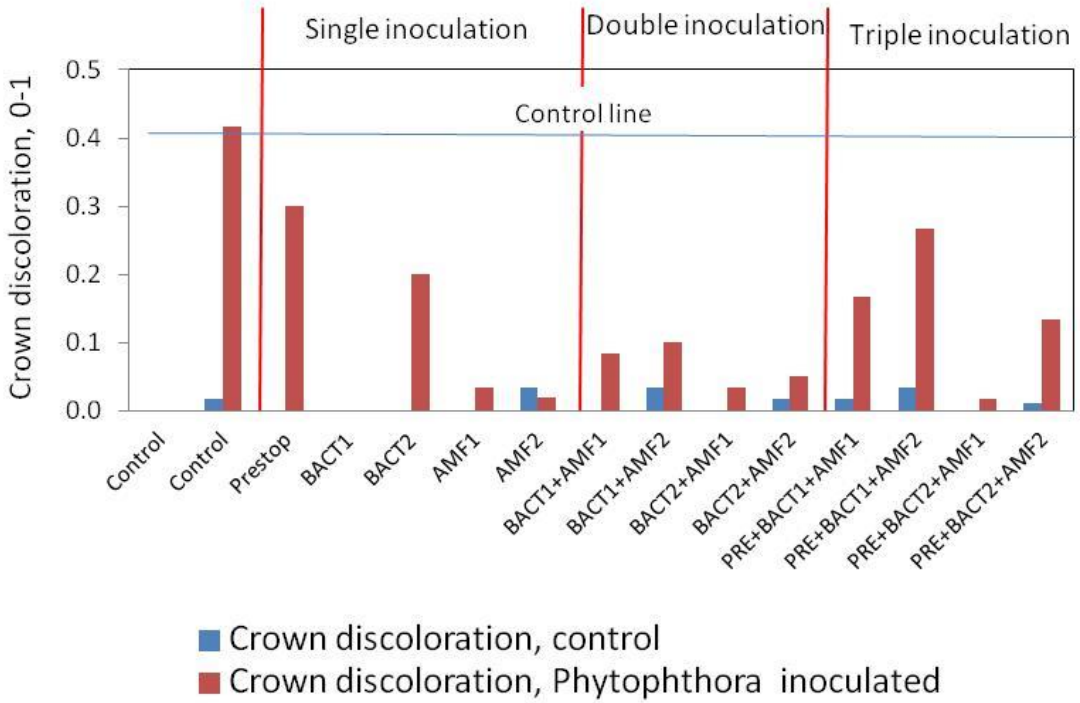
Root health




- Root health seemed to be increased by most of the microbial treatments.
- In several cases root health of *Phytophthora* inoculated plants was higher than of non-inoculated!?
- Results can be regarded as unreliable as the difference in root health was very small also in the control!

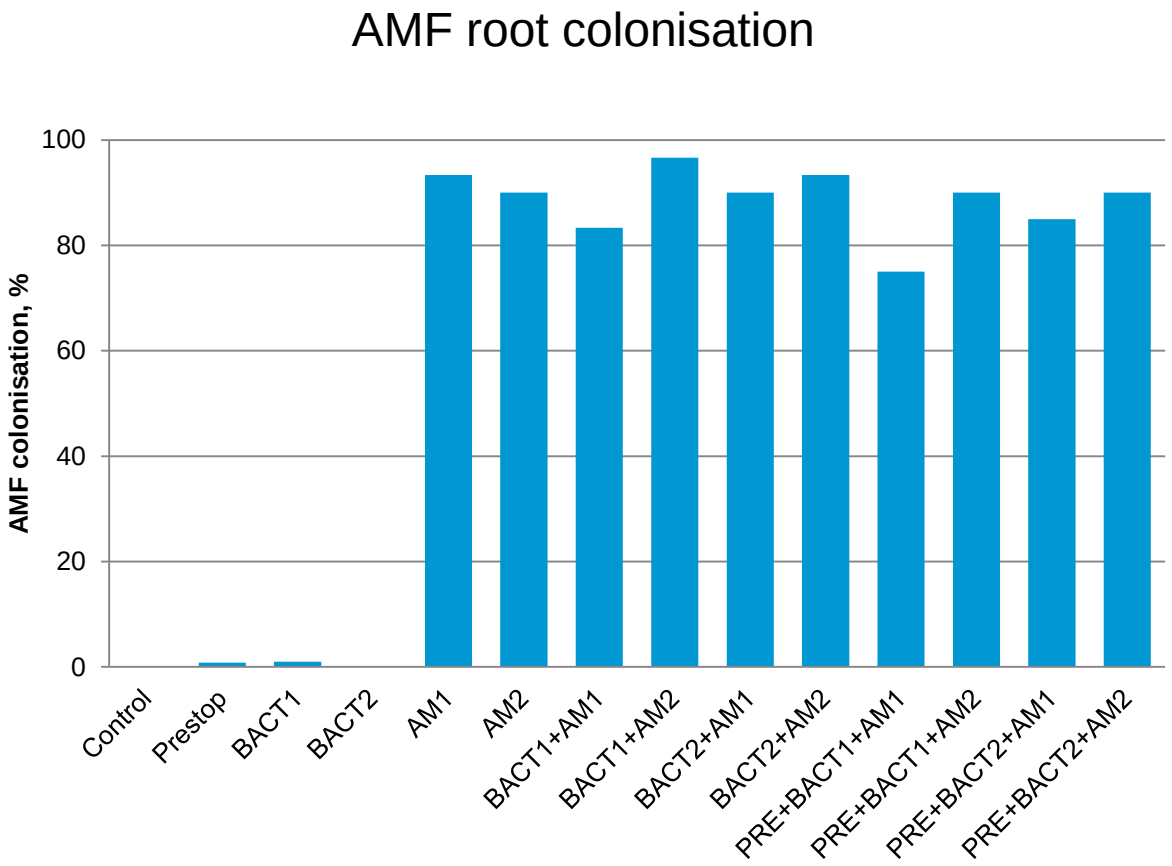
# Results 2012 – AMF and *Pseudomonas*

Crown discoloration




- Most microbial treatments caused a decrease in crown discoloration as compared with the control
- No difference between single, double or triple microbial inoculation
- Most promising treatments were BACT1, AMF1, AMF2, BACT2+AMF1, BACT2+AMF2 AND PRE+BACT2+AMF1

# Results 2012 – AMF and *Pseudomonas*




- Strawberry roots were well infected by AM fungi. Colonisation was very similar for single, dual or triple microbial inoculation
- **No negative interactions occurred between microorganisms**

# Summary of experiment 2/2012

+ = positive impact, 0 = no impact, — = negative impact

| Treatment | Shoot DW | Shoot vigor | Crown discoloration |
|---|---|---|---|
| Prestop | 0 | 0 | 0 |
| BACT1 | 0 | + | + |
| BACT2 | + | 0 | 0 |
| AMF1 | 0 | + | + |
| AMF2 | 0 | 0 | + |
| BACT1+AMF1 | + | + | + |
| BACT1 + AMF2 | + | + | + |
| BACT2+AMF1 | + | + | + |
| BACT2+AMF2 | + | + | + |
| PRE+BACT1+AMF1 | + | 0 | 0 |
| PRE+BACT1+AMF2 | + | 0 | 0 |
| PRE+BACT2+AMF1 | — | + | + |
| PRE+BACT2+AMF2 | + | 0 | 0 |

- There is a better possibility of controlling strawberry crown rot when using microbial mixtures than with single microorganisms.

# GENERAL CONCLUSIONS

- Studying the effects of microbial inoculation against strawberry crown rot in pot experiments was difficult because of problems in managing disease severity. Crown rot severity is influenced by growing medium, light conditions, type of plant material, inoculation method…..

- After various kinds of optimization experiments, AMF and endophytic *Pseudomonas* were successfully tested together in 2012.

- Microbes or their mixtures did not control *P. cactorum completely* in pot experiments, but there seems to be a better chance of a achieving this goal by using double microbial inoculation instead of single inoculation.

- Further experiments need to be conducted in which should be studied
  - Effect of microorganis in relation to chemical control
  - Effect of microorgnisms in true disease situation in the field

# Thank Your for Your Attention!

We did this: