IPM 2.0
Towards future-proof crop protection in Europe

UNIVERSITEIT GENT

FACULTY OF BIOSCIENCE ENGINEERING

Influence of soil substrate on the biocontrol capacity of *Pseudomonas* CMR12a against Rhizoctonia root rot on bean

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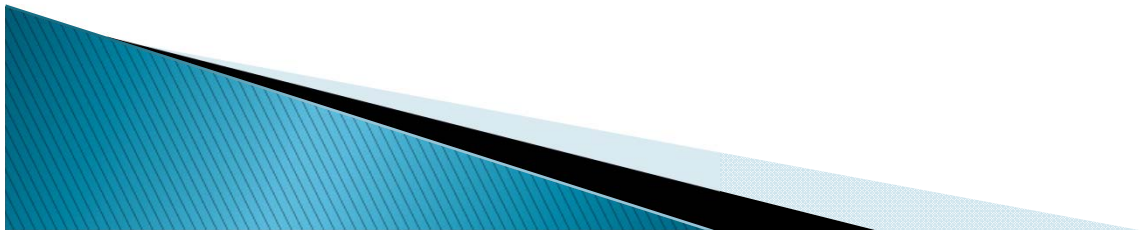
EUROPEAN FOUNDATION FOR PLANT PATHOLOGY
EFPP

Outline

- Introduction
- Methodology
- Results and Discussion
- Conclusions
- Future prospectives



Introduction

Beans (*Phaseolus vulgaris* L.)

- Most important grain legumes for direct human consumption in the world.

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- Most important grain legumes for direct human consumption in the world.

- **Health benefits from consuming beans on a regular basis:**
  - Maintenance of a healthy weight
  - Reduced risk of diabetes
  - Reduced risk of heart disease
  - Reduced risk of colon cancer

Introduction

Rhizoctonia solani

- Very common soil-borne pathogen

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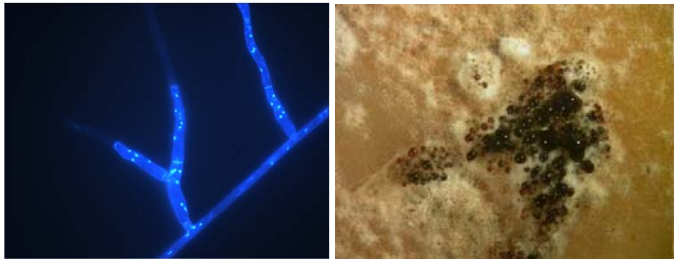
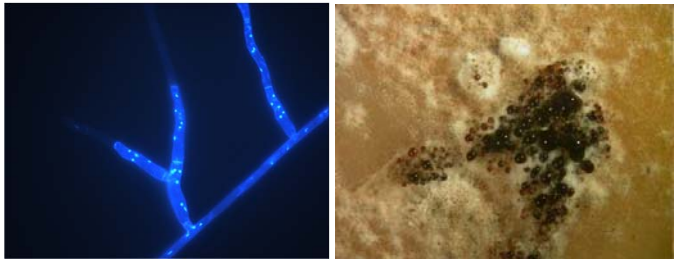


- Exists primarily as
  - Mycelium
  - Sclerotia

Introduction

Rhizoctonia solani

- Very common soil-borne pathogen



- Exists primarily as
  - Mycelium
  - Sclerotia

- Great diversity of host plants

Introduction

Root rot on Bean

- Occur on young seedlings

- Small, oval to elliptical, reddish-brown sunken lesions or cankers on stem and roots

- Severely infected seedlings = dead

Research goal

Determining influence of different soil-sand mixtures on biocontrol capacity of *Pseudomonas* CMR12a against Rhizoctonia root rot

Introduction

- **Biocontrol agents:**
  - *Pseudomonas* CMR12a
  - CMR12a-mutants

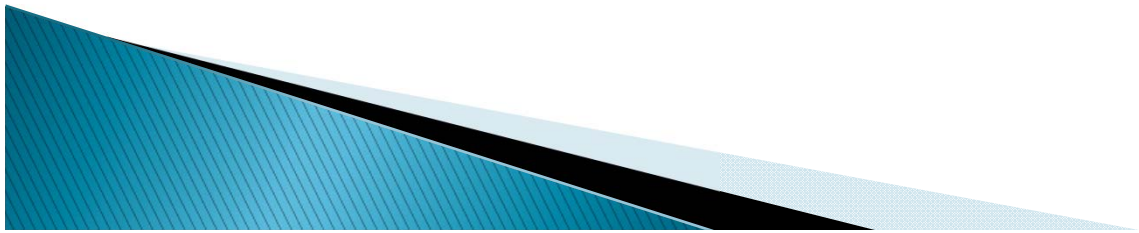
???

Introduction

- **Biocontrol agents:**
  - *Pseudomonas* CMR12a
  - CMR12a-mutants
- **Background knowledge:**
  - Non-pathogenic on bean
  - Able to produce important antibiotics:
    - Phenazines (Phz)
    - Cyclic lipopeptides (CLPs)
  - Successful biocontrol agent

Biological Control of Rhizoctonia Root Rot on Bean by Phenazine- and Cyclic Lipopeptide-Producing *Pseudomonas* CMR12a

Jolien D'aes, Gia Khuong Hoang Hua, Katrien De Maeyer, Joke Pannecoucque, Ilse Forrez, Marc Ongena, Lars E. P. Dietrich, Linda S. Thomashow, Dmitri V. Mavrodi, and Monica Höfte



In vivo biocontrol of Rhizoctonia root rot on bean

- **Soil substrates:**
  - 25% potting soil : 75% sand
  - 50% potting soil : 50% sand
  - 75% potting soil : 25% sand

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- **Fungal isolate:**
  - AG 2-2 18

In vivo biocontrol of Rhizoctonia root rot on bean

- **Soil substrates:**
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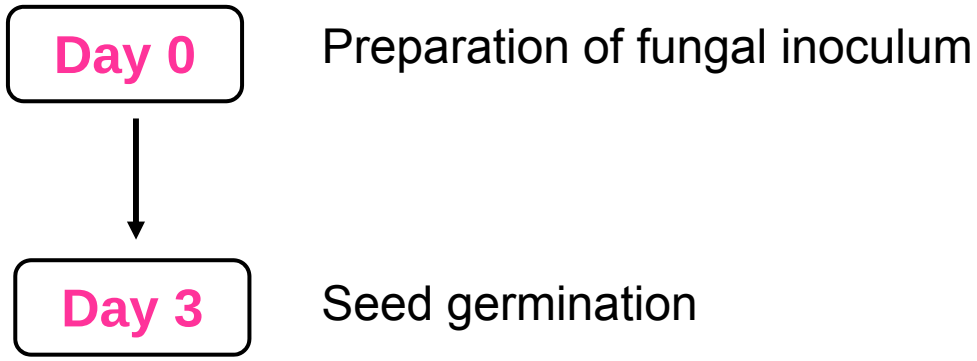
  - 75% potting soil : 25% sand
- **Fungal isolate:**
  - AG 2-2 18
- **Bacterial isolates:**
  - CMR12a (Phz^+ and $CLP1^+$)
  - CMR12a-ΔPhz (Phz^- and $CLP1^+$)
  - CMR12a-CLP1 (Phz^+ and $CLP1^-$)
  - CMR12a- ΔPhz-CLP1 (Phz^- and $CLP1^-$)

In vivo biocontrol of Rhizoctonia root rot on bean

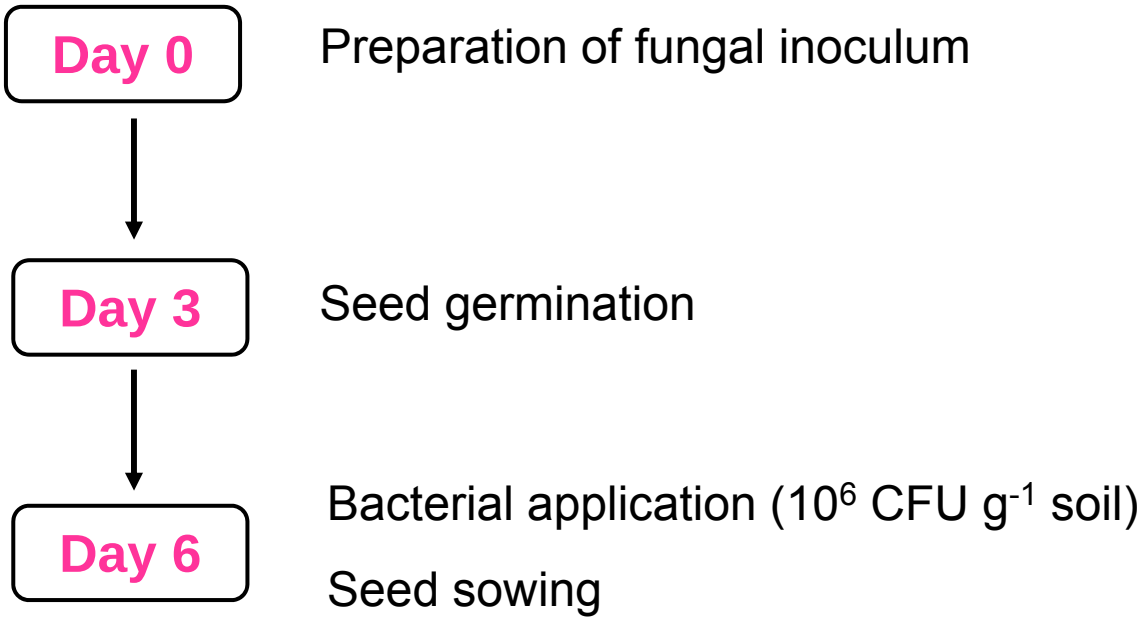
Day 0

Preparation of fungal inoculum

In vivo biocontrol of Rhizoctonia root rot on bean



In vivo biocontrol of Rhizoctonia root rot on bean



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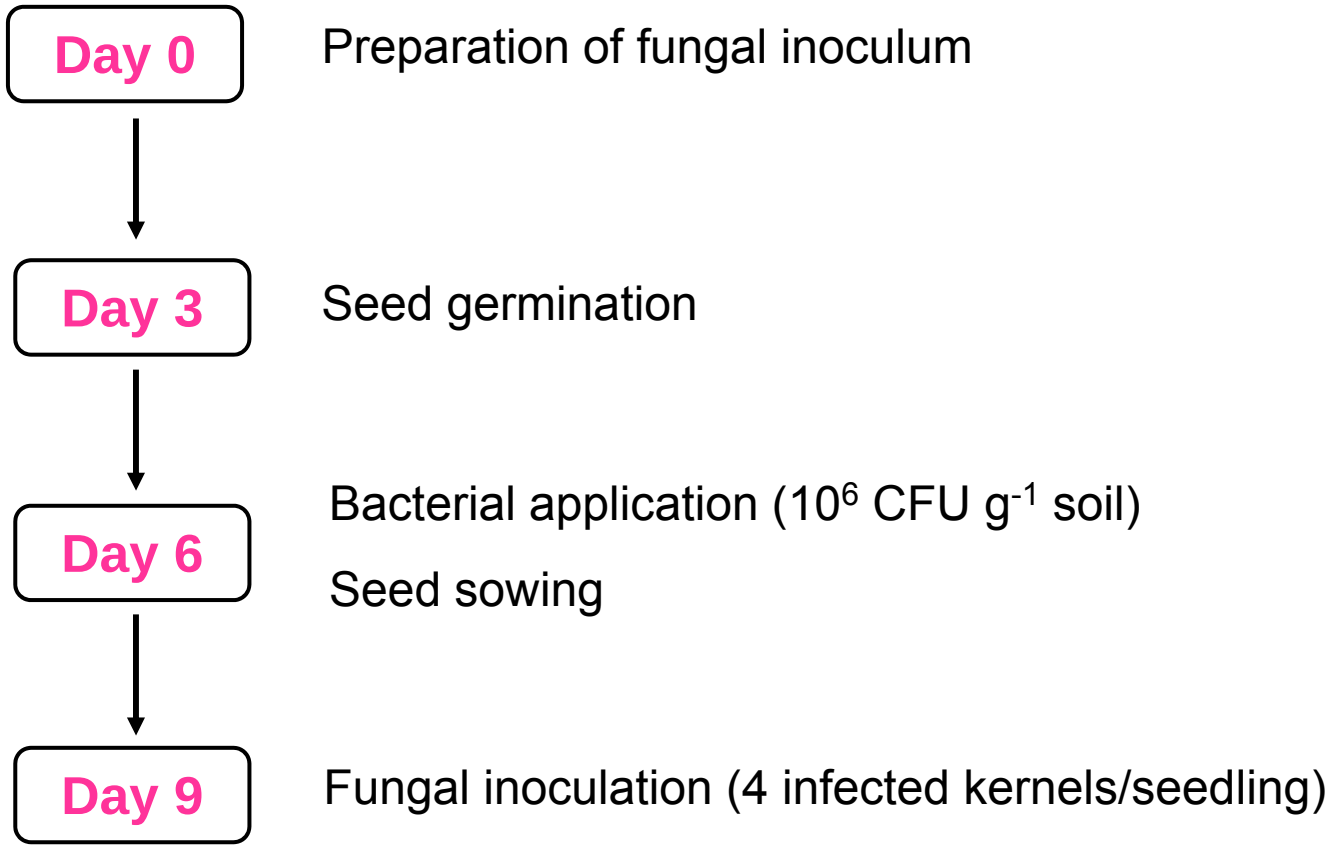

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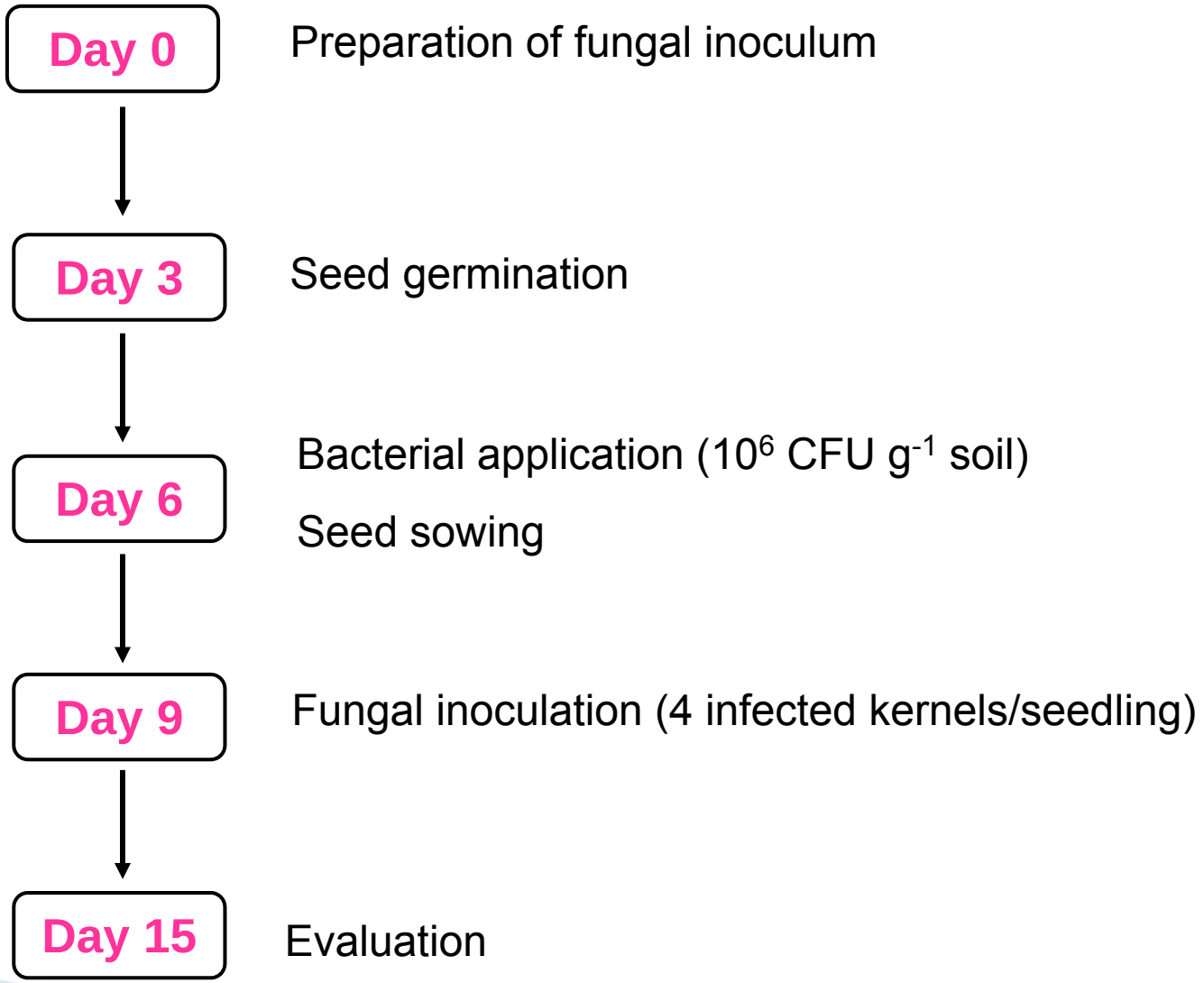


In vivo biocontrol of Rhizoctonia root rot on bean

Class 0: Healthy, no symptoms observed

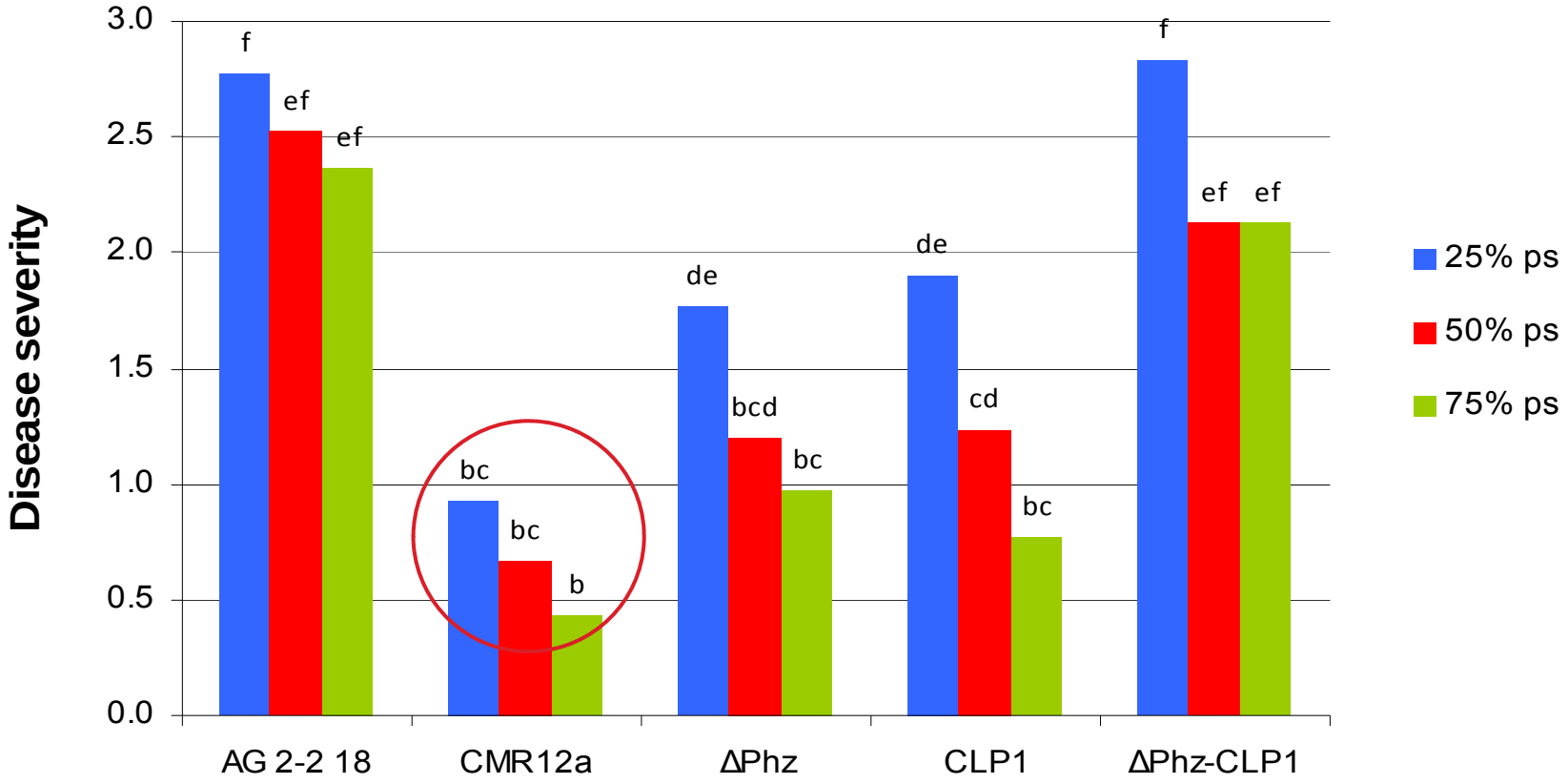
1: Lesion ≤ 25% of stem and/or hypocotyl

2: Lesion ≤ 50% of stem and/or hypocotyl

3: Lesion ≤ 75% of stem or hypocotyl

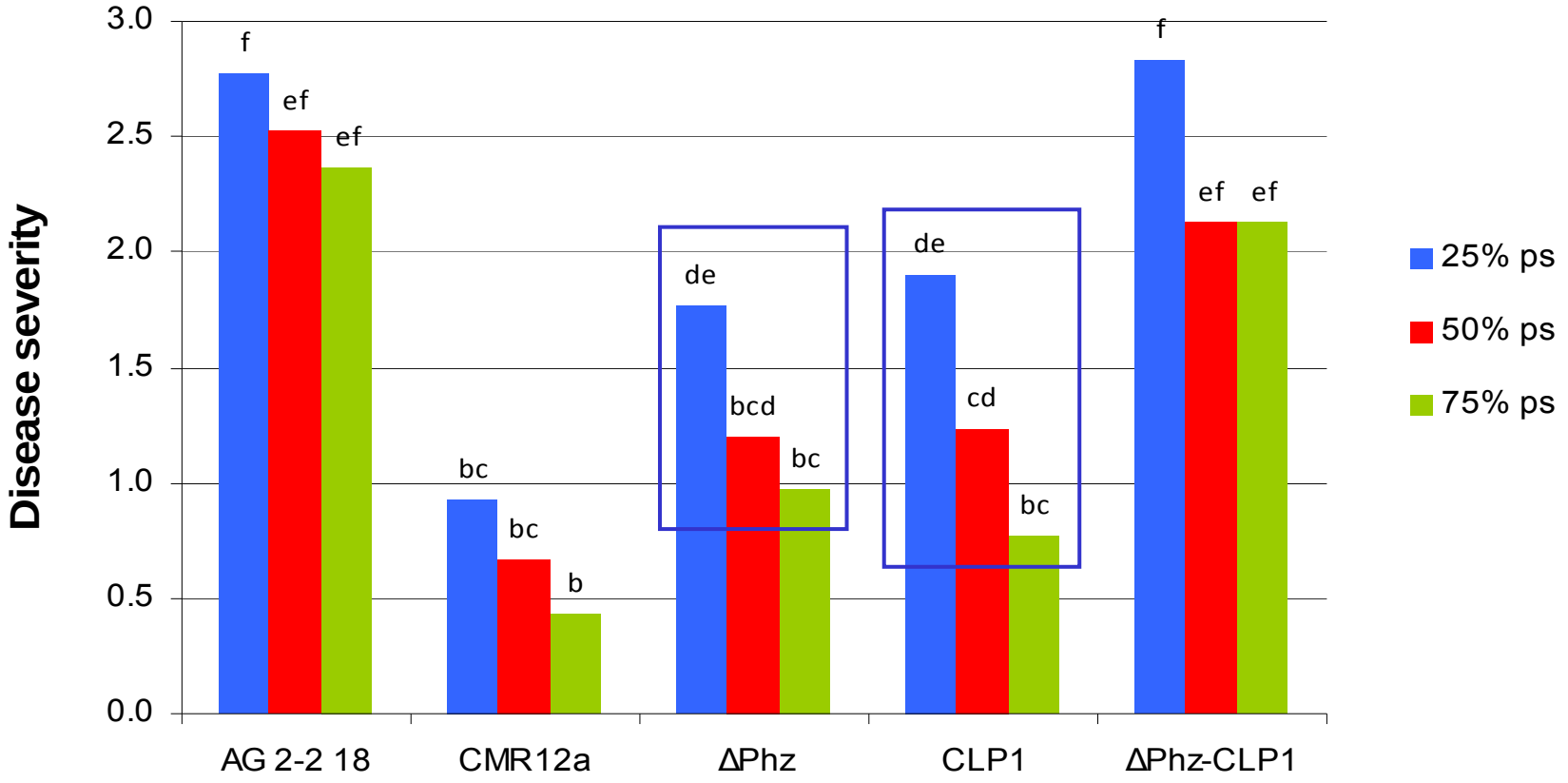
4: Hypocotyl is completely decayed and seedling dead

In vivo biocontrol of Rhizoctonia root rot on bean



Phz	+	-	+	-
CLP1	+	+	-	-

In vivo biocontrol of Rhizoctonia root rot on bean



Phz	+	-	+	-
CLP1	+	+	-	-

Influence of soil substrates on the growth of bean seedlings

Day 0

- Seed sowing
- Soil substrates used:
  - 25% potting soil: 75% sand
  - 50% potting soil: 50% sand
  - 75% potting soil: 25% sand

Influence of soil substrates on the growth of bean seedlings

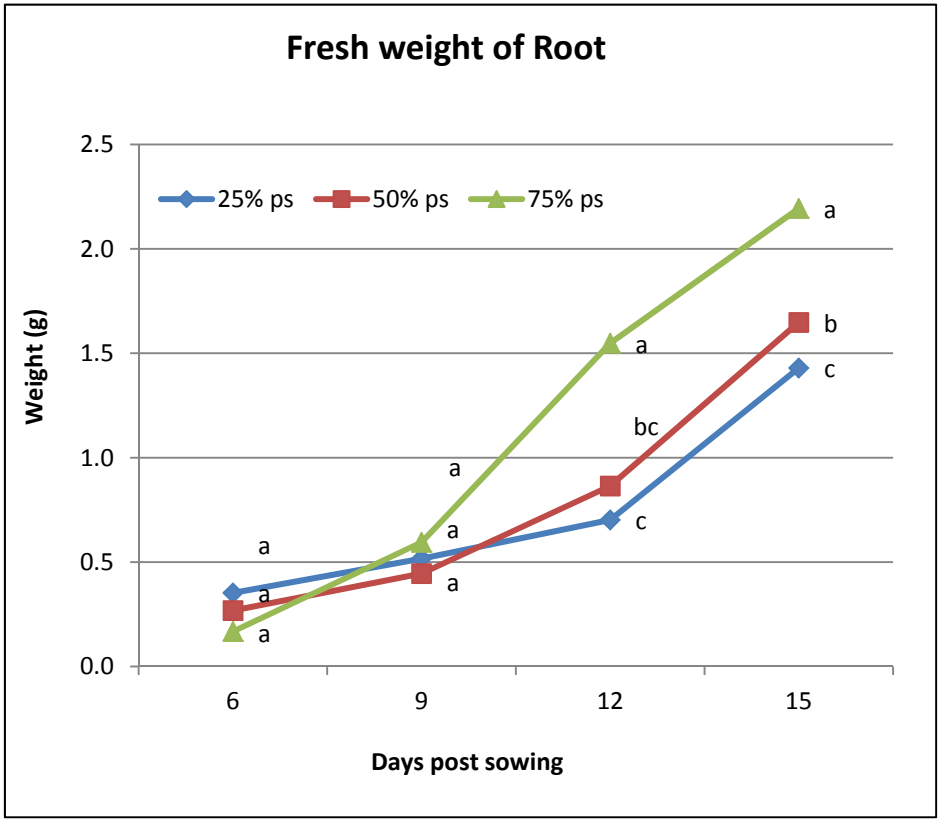
Day 0 → **Day 6, 9 and 15**

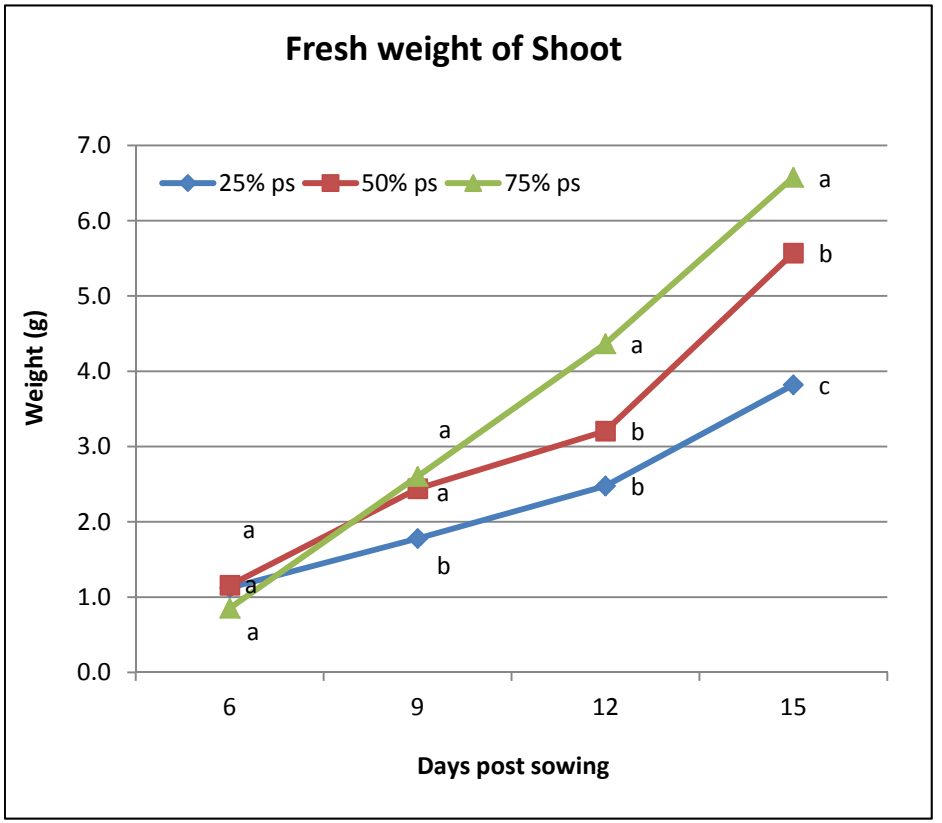
- Seed sowing
- Soil substrates used:
  - 25% potting soil: 75% sand
  - 50% potting soil: 50% sand
  - 75% potting soil: 25% sand

Data record:

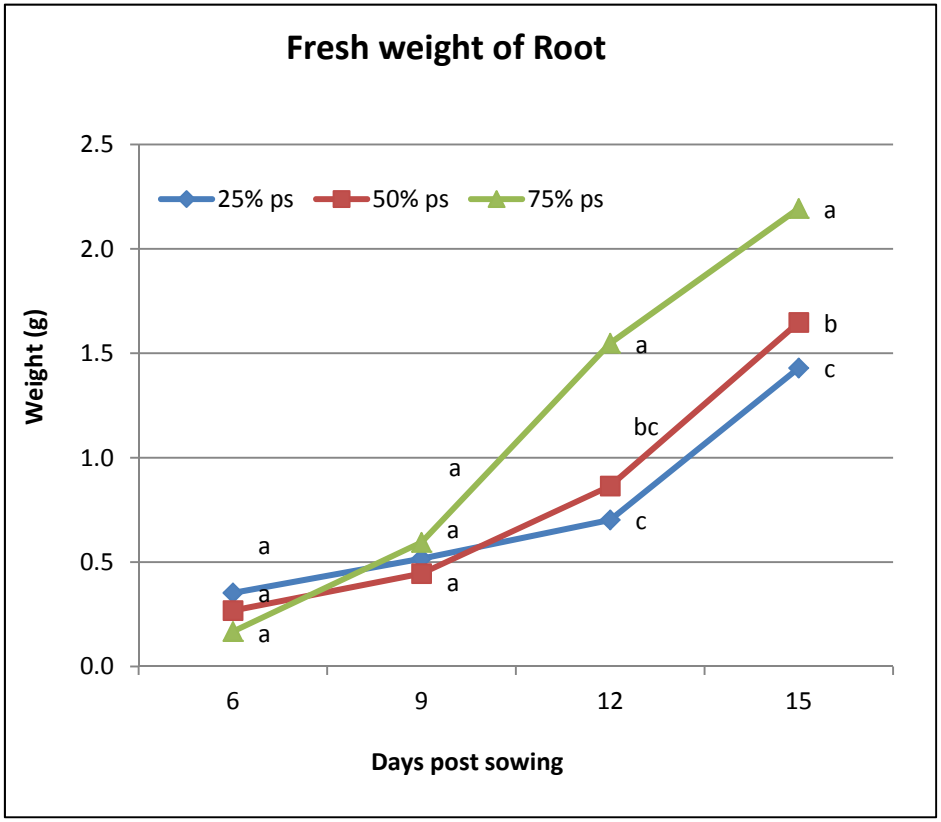
- Diameter of hypocotyl
- Length of shoot and root
- Fresh and dry weight of shoot and root

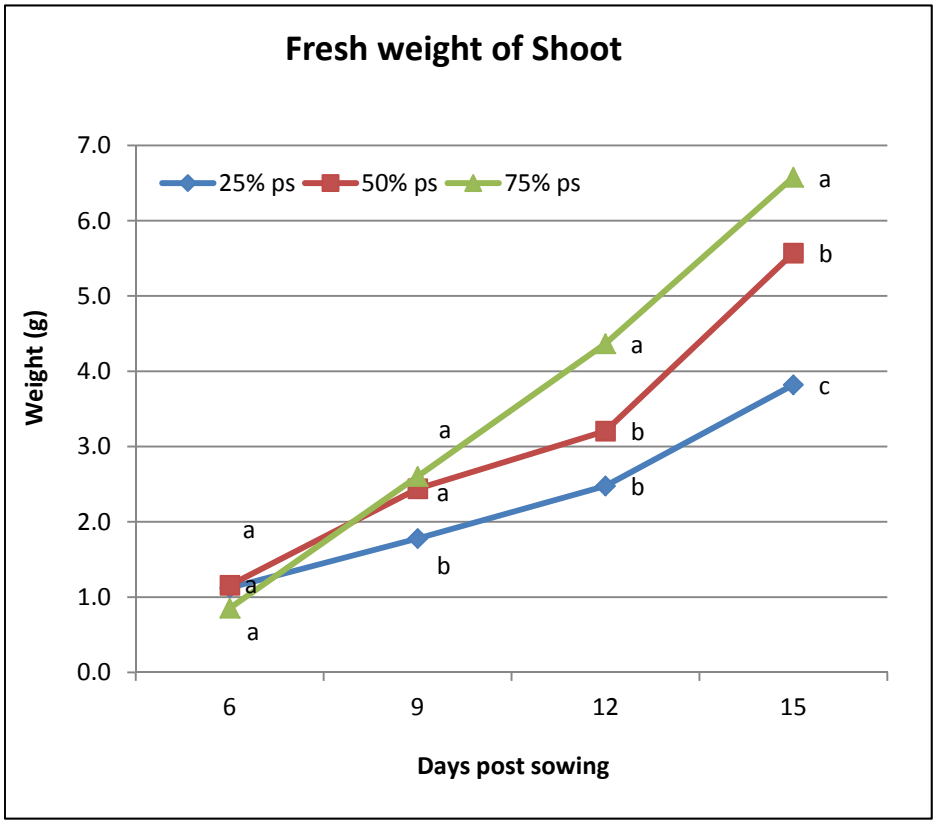
Influence of soil substrates on the growth of bean seedlings





Influence of soil substrates on the growth of bean seedlings





- Optimal growth was observed in substrate containing 75% potting soil
- The decrease in proportion of potting soil results in the decrease in seedling growth

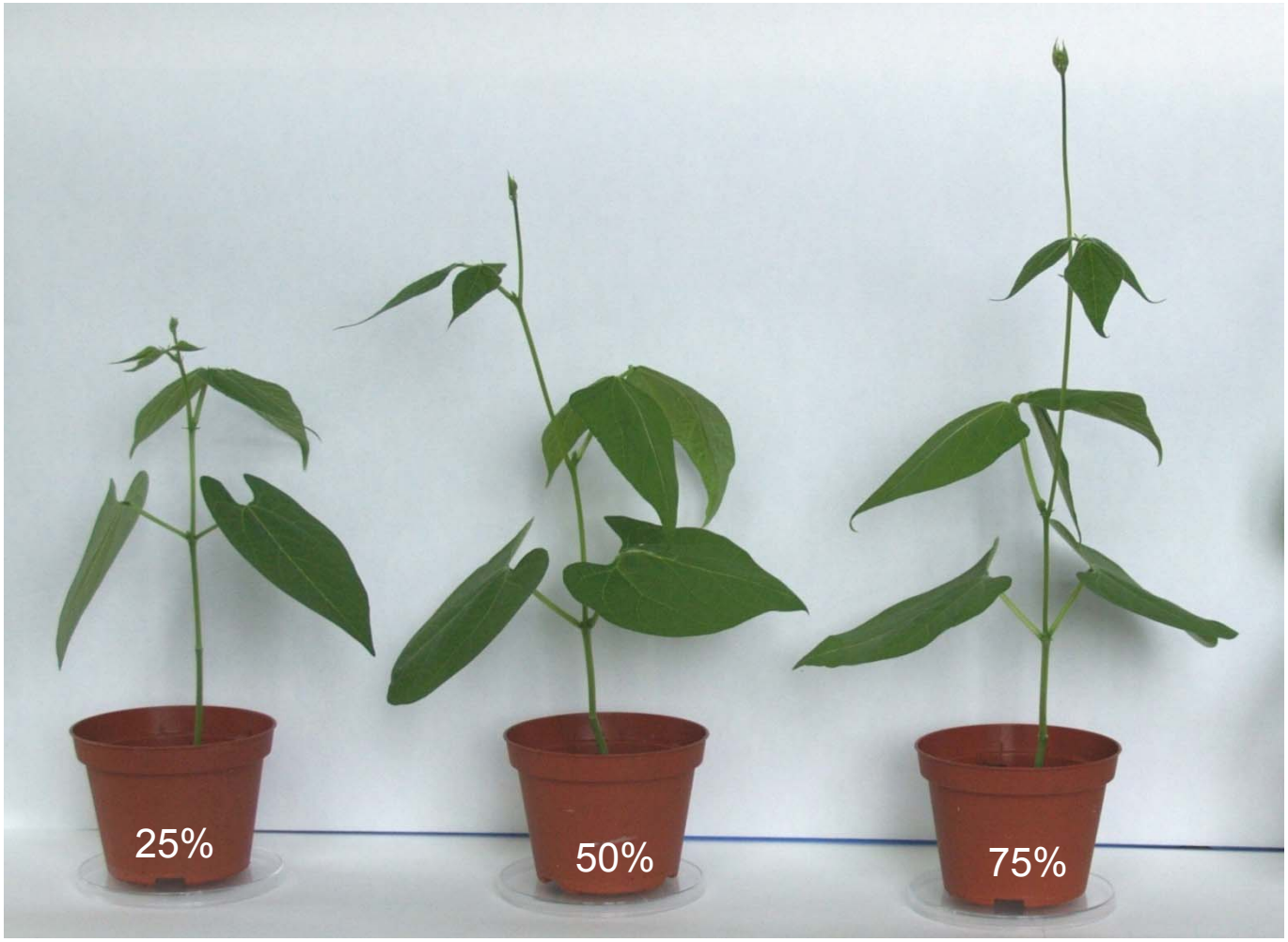


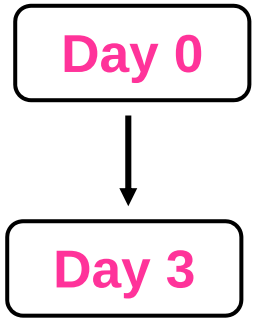
Fig 1. Difference in the development of bean seedlings grown on substrates containing various percentage of potting soil

Influence of soil substrates on the spread of *Rhizoctonia solani*

Day 0

Preparation of fungal inoculum

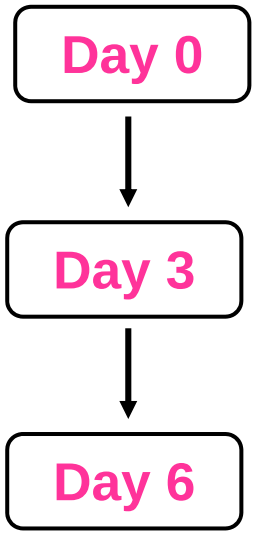
Influence of soil substrates on the spread of *Rhizoctonia solani*



Preparation of fungal inoculum

Seed germination

Influence of soil substrates on the spread of *Rhizoctonia solani*



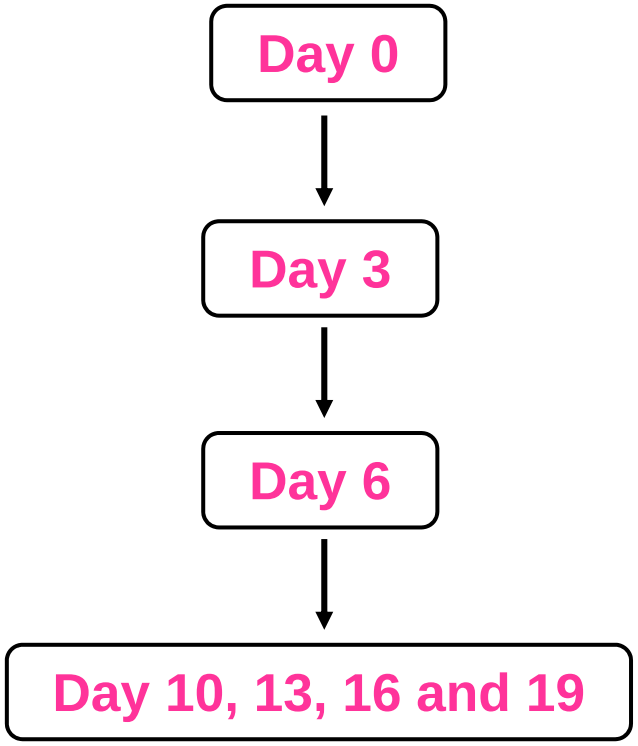
Preparation of fungal inoculum

Seed germination

Seed sowing

Fungal inoculation (1 infected kernel/seedling)

Influence of soil substrates on the spread of *Rhizoctonia solani*



Preparation of fungal inoculum

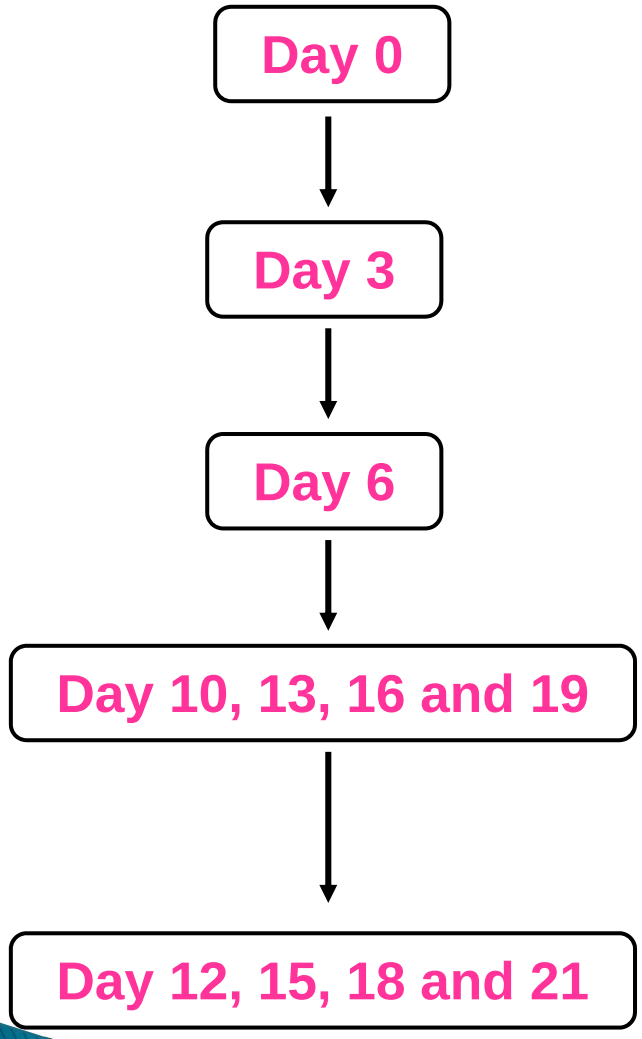
Seed germination

Seed sowing

Fungal inoculation (1 infected kernel/seedling)

Inserting sterile toothpicks along the seedling rows

Influence of soil substrates on the spread of *Rhizoctonia solani*



Preparation of fungal inoculum

Seed germination

Seed sowing

Fungal inoculation (1 infected kernel/seedling)

Inserting sterile toothpicks along the seedling rows

Removing the toothpicks from soil

Placing toothpicks on Rhizoctonia selective medium

Determining the number of toothpicks colonized by *Rhizoctonia* hyphae

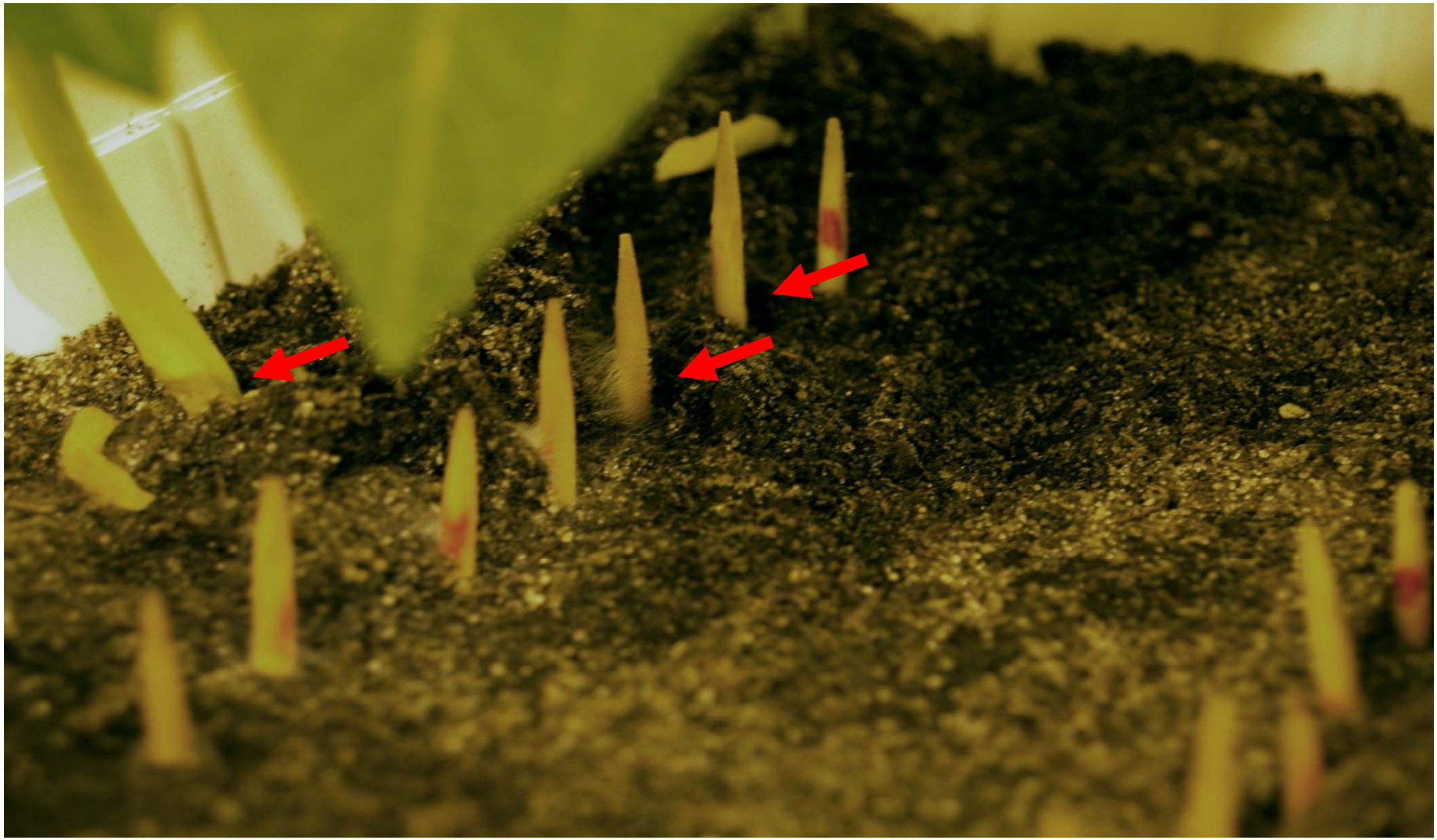
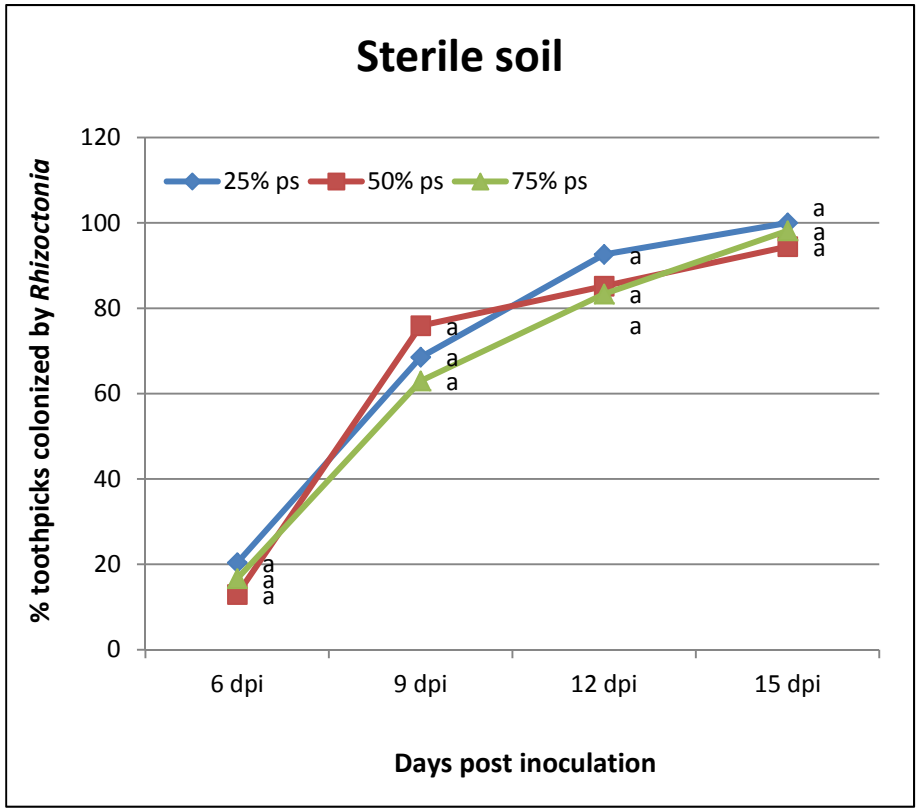
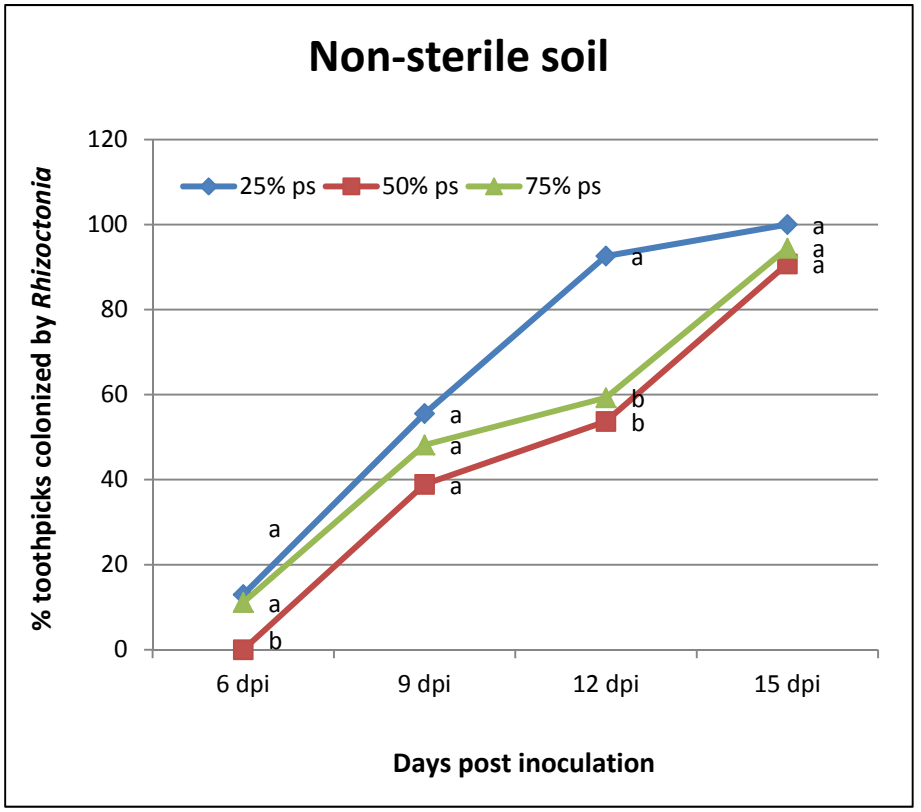


Fig 2. Correspondence between the colonization of *R. solani* on toothpicks and the appearance of disease symptoms on bean seedlings

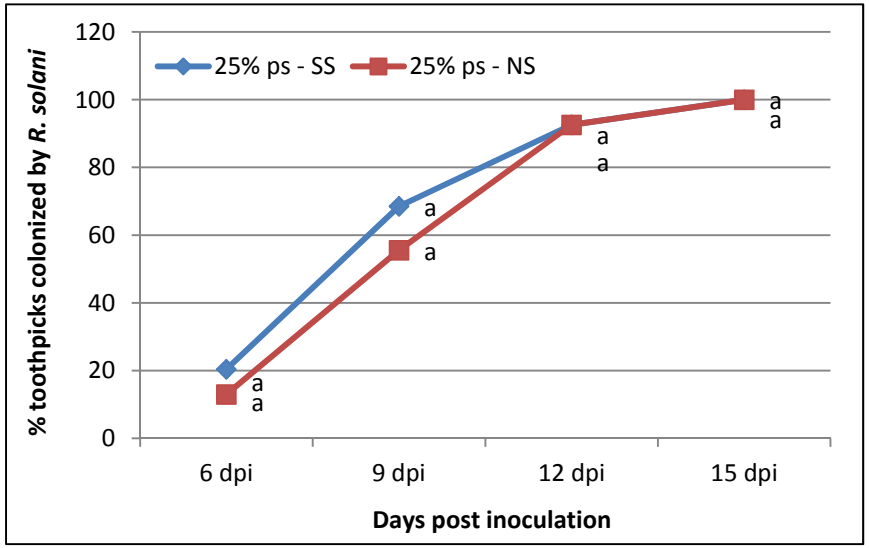
Influence of soil substrates on the spread of *Rhizoctonia solani*

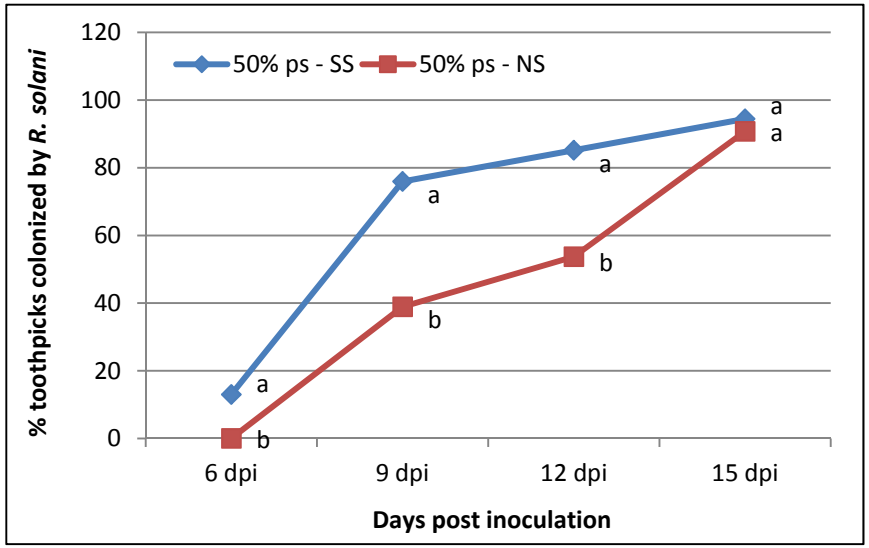


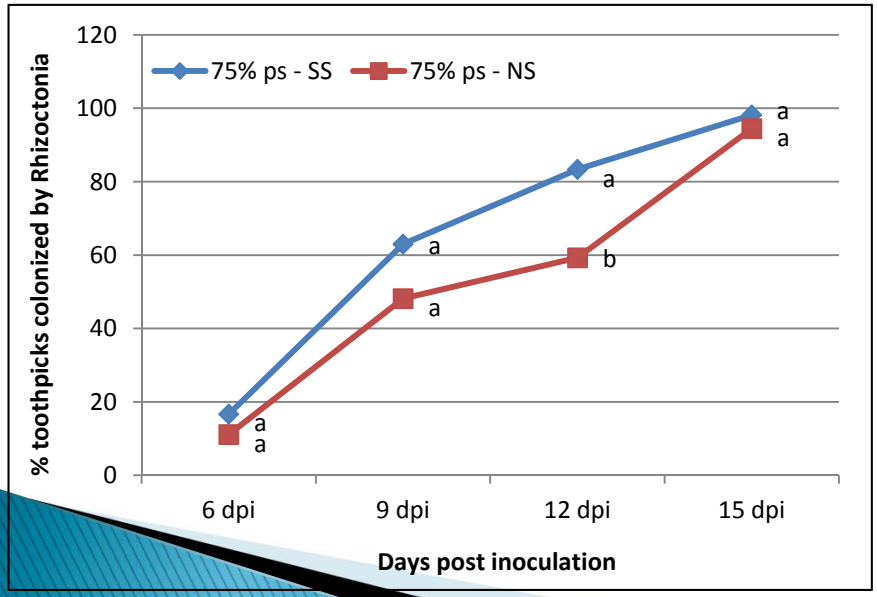


- **Sterile soil:** no significant difference amongst treatments
- **Non-sterile soil:** fastest invasion was observed in substrate with 25% ps

Influence of soil substrates on the spread of *Rhizoctonia solani*

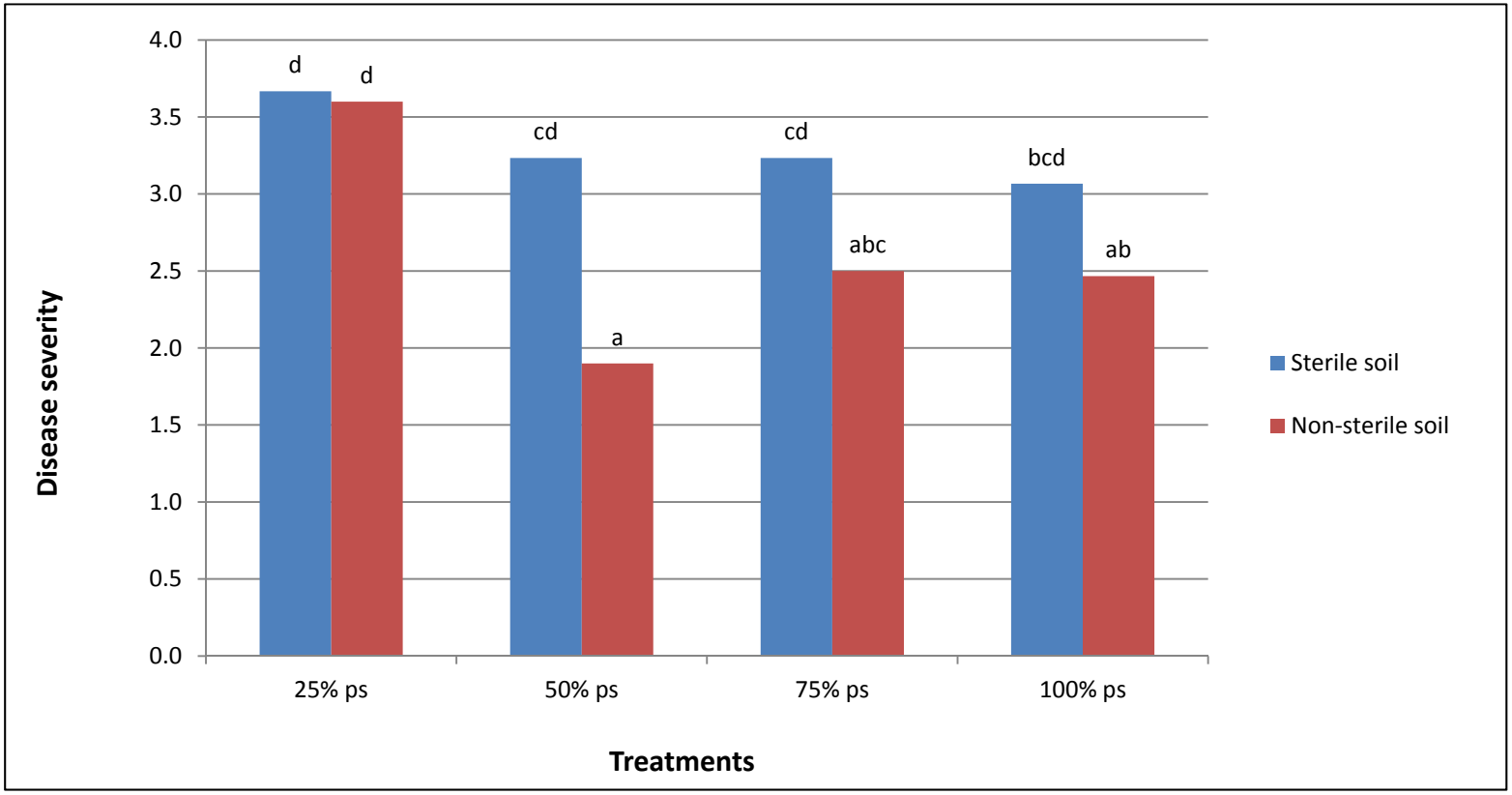




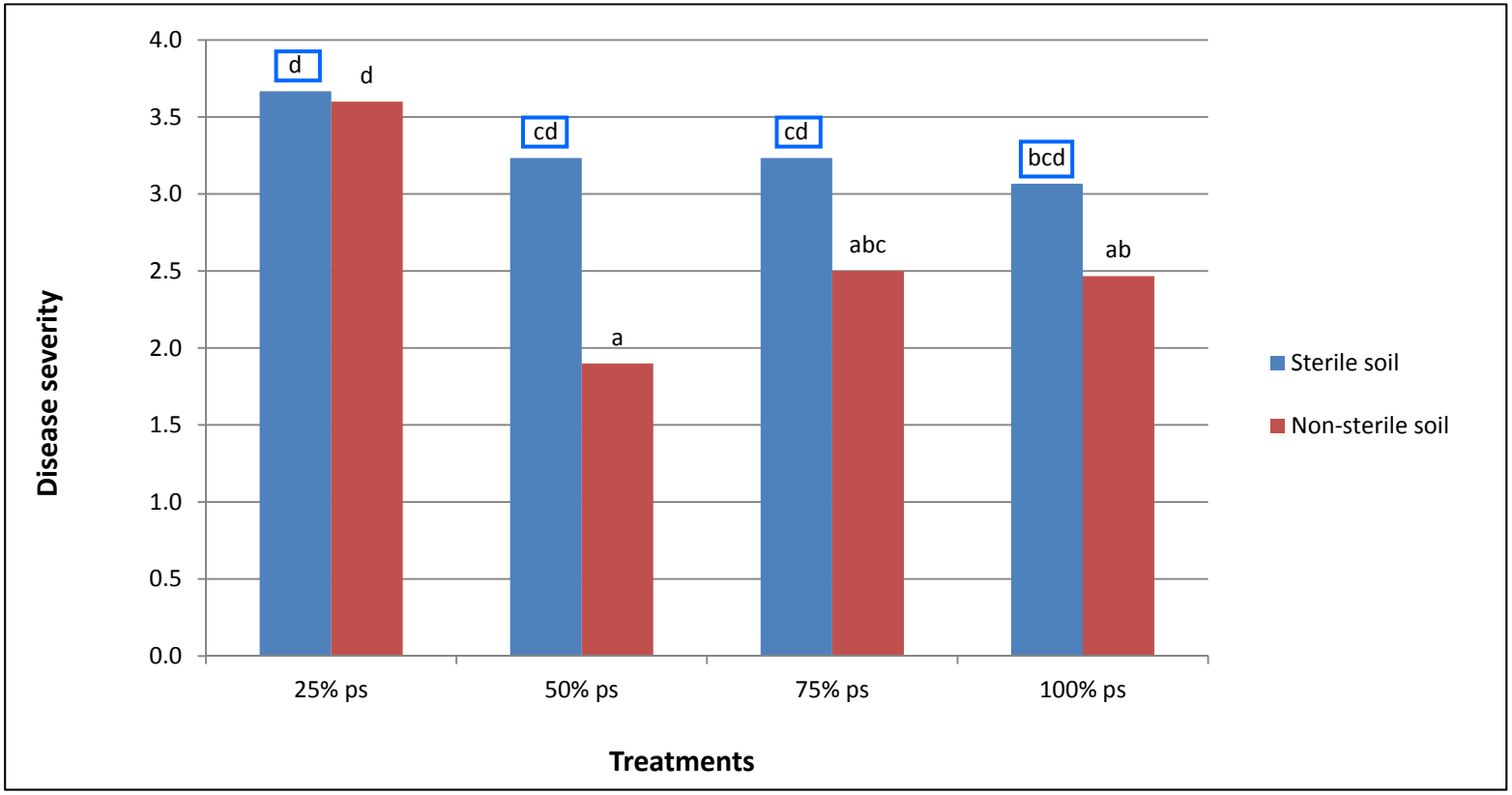


Rhizoctonia hyphae spreaded slower in non-sterile soil

Influence of soil substrates on the spread of *Rhizoctonia solani*

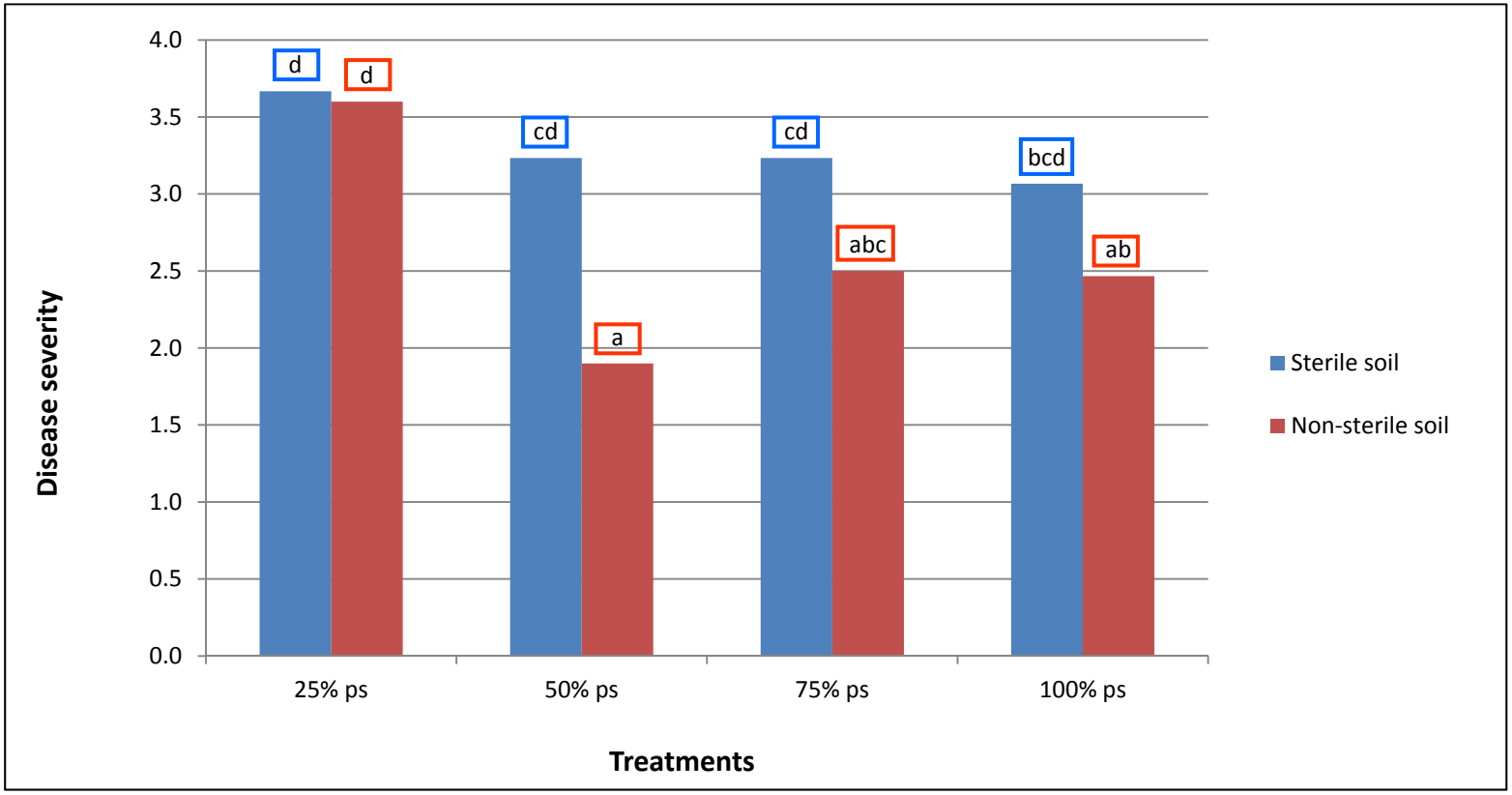


Influence of soil substrates on the spread of *Rhizoctonia solani*



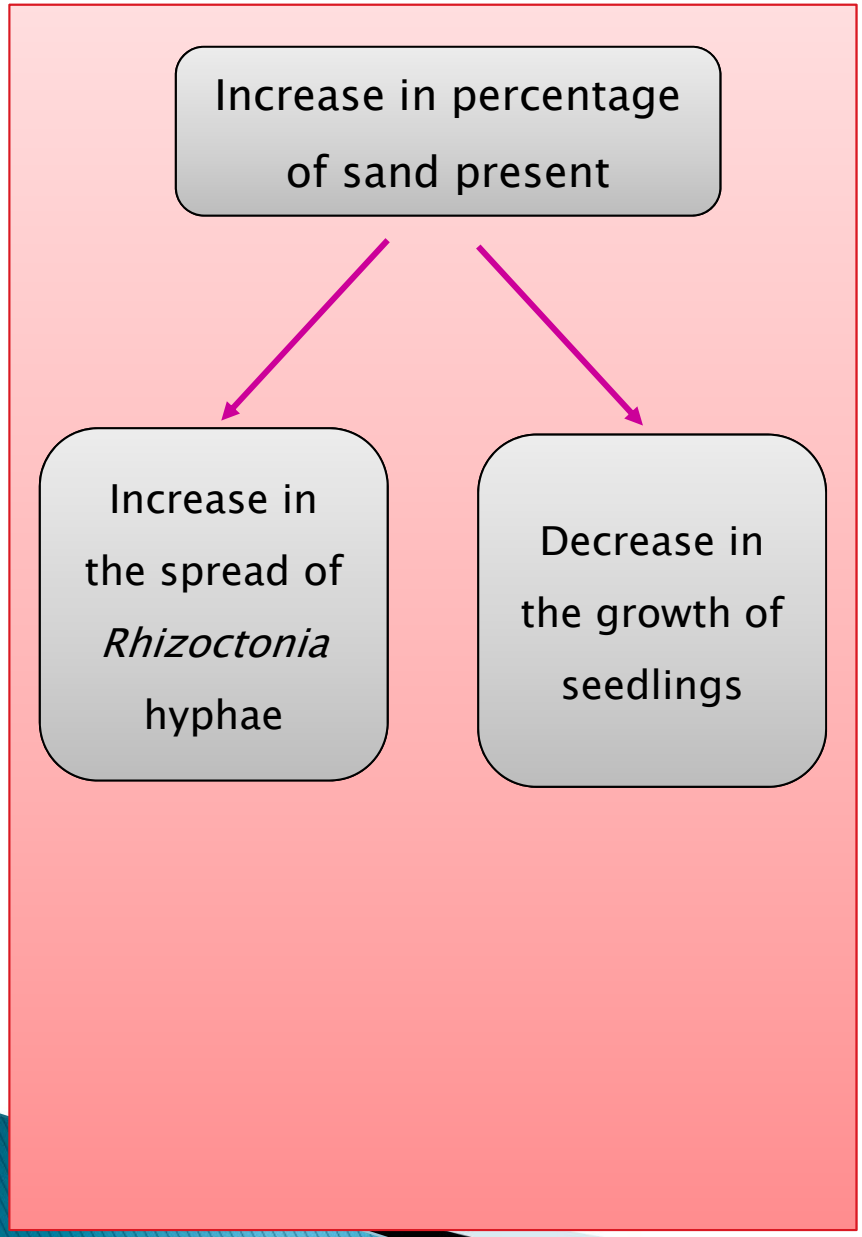
- **Sterile soil:** no significant difference amongst soils

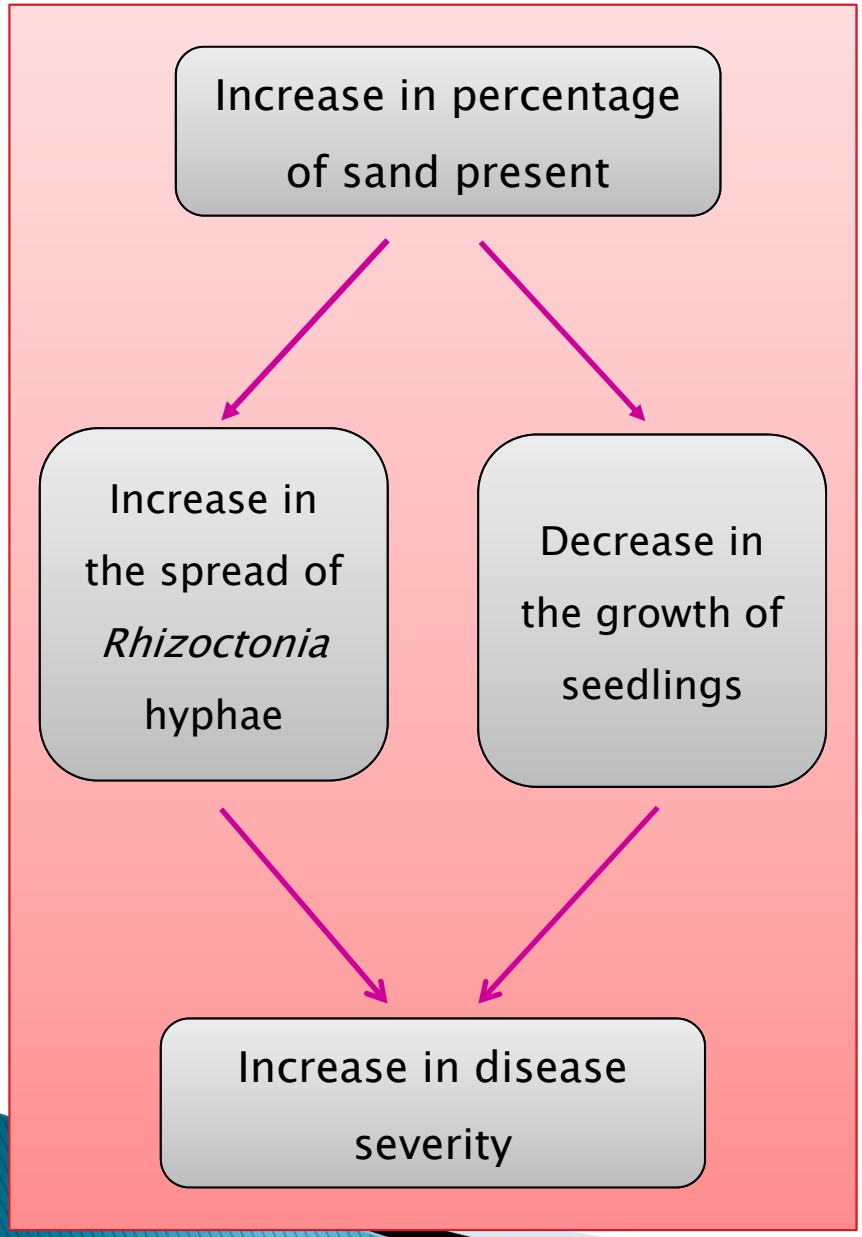
Influence of soil substrates on the spread of *Rhizoctonia solani*

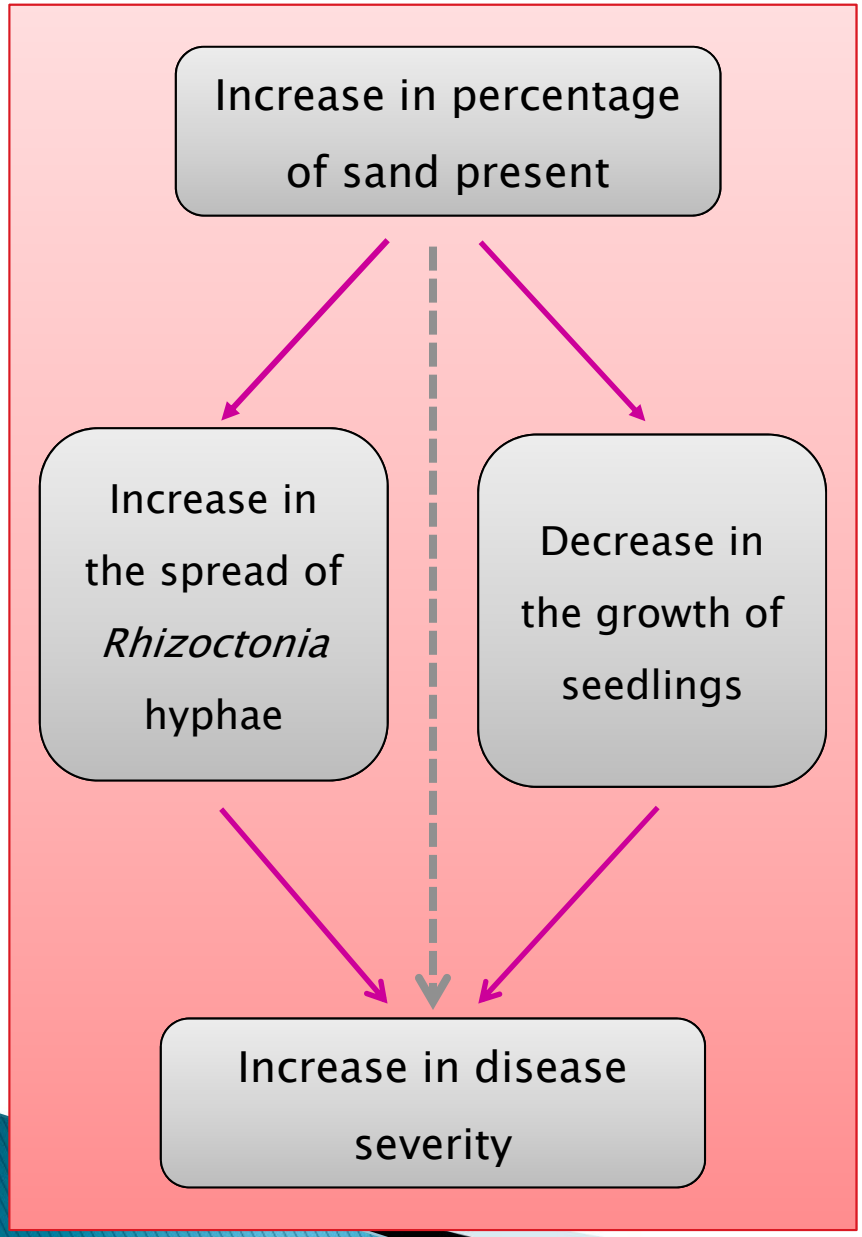


- **Sterile soil:** no significant difference amongst soils
- **Non-sterile soil:** disease severity was highest in 25% ps and lowest in 50% ps

Increase in percentage of sand present


Increase in percentage of sand present
Increase in the spread of *Rhizoctonia* hyphae
Decrease in the growth of seedlings


Increase in percentage of sand present
Increase in the spread of *Rhizoctonia* hyphae
Decrease in the growth of seedlings
Increase in disease severity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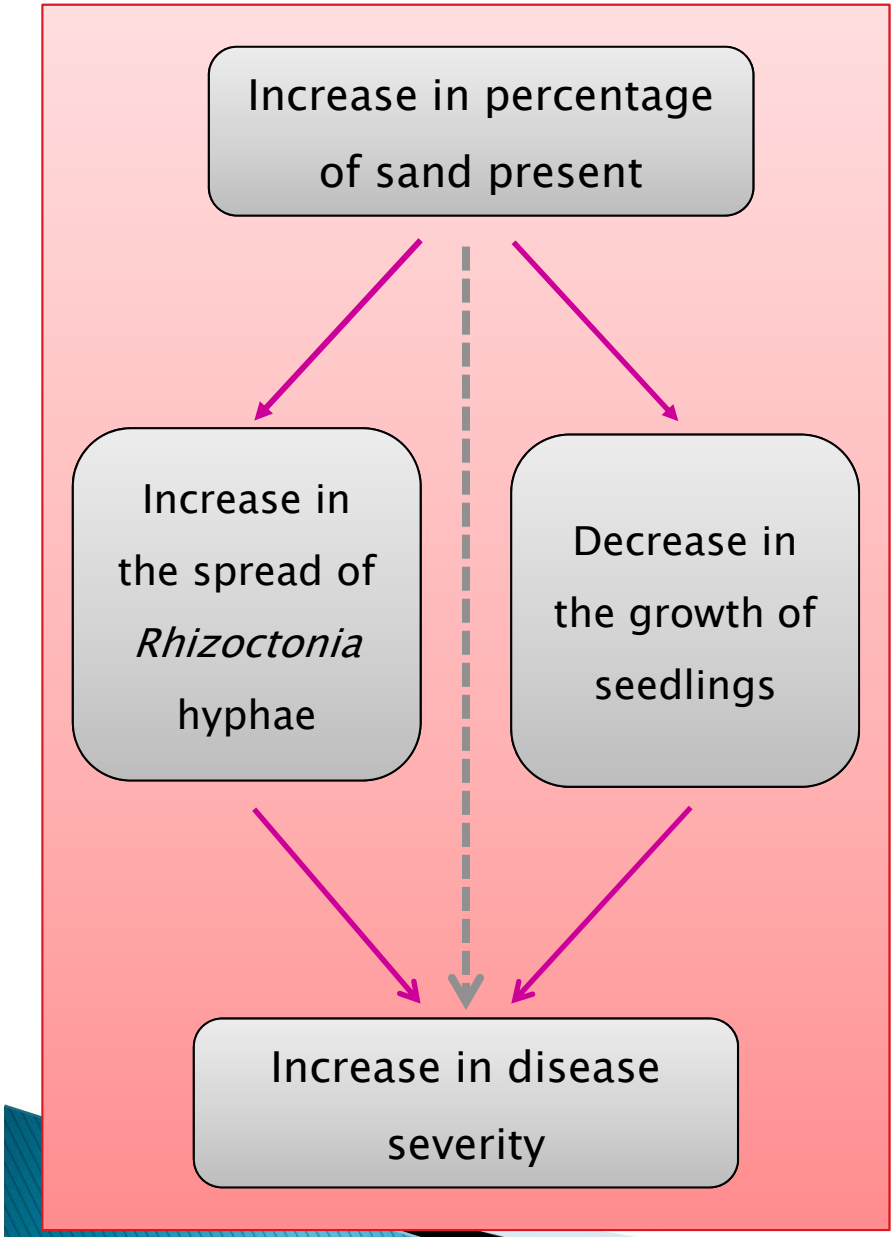
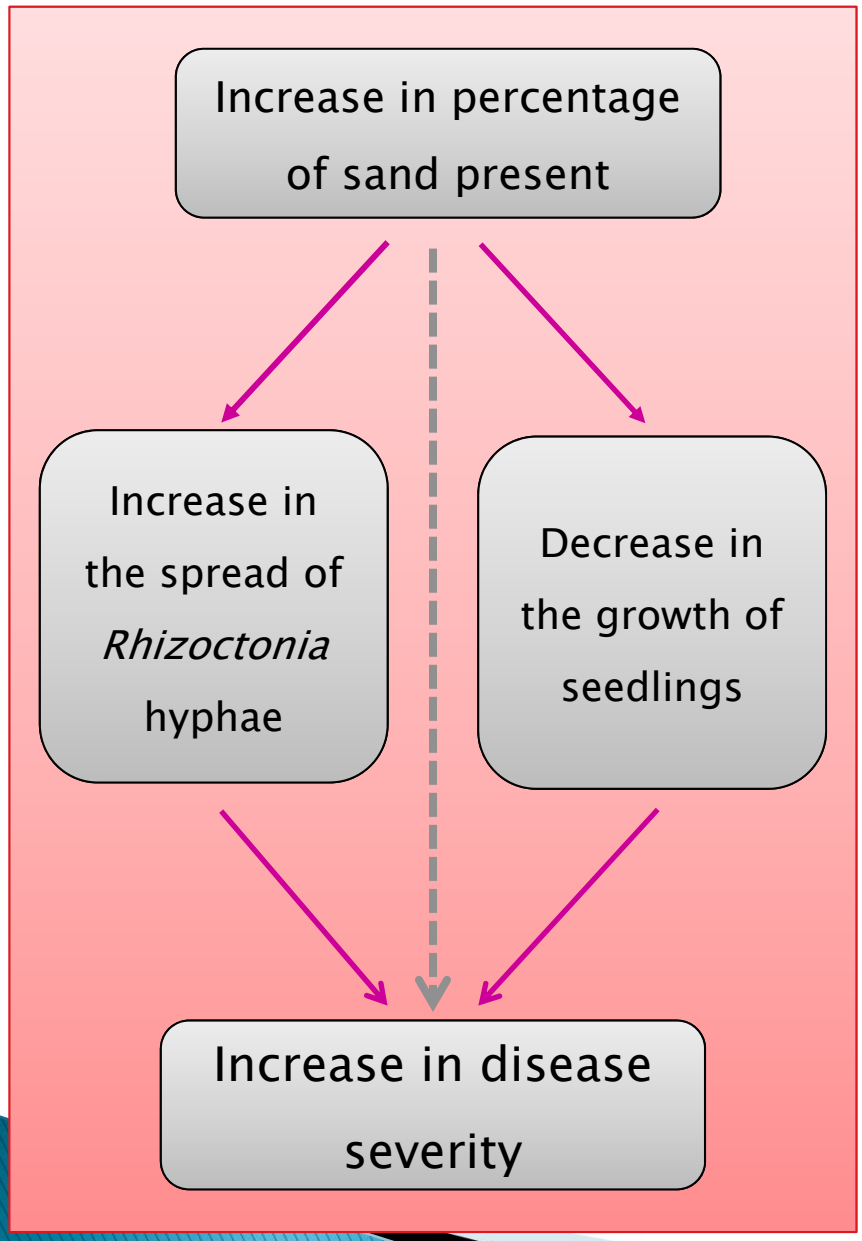


75% potting soil

50% potting soil

25% potting soil

Phz or CLPs alone is sufficient



75% potting soil

50% potting soil

Phz or CLPs alone is sufficient

25% potting soil

Phz and CLPs are required to be effective

Future Prospects

- Studying the survival and multiplication capacity of CMR12a and CMR12a-mutants in different soil substrates
- Analysing the physical and chemical characteristics of soil combinations used.
- Exploring induced systemic resistance capacity of phenazines and cyclic lipopeptides

Acknowledgements

- Prof. Monica Höfte
- Katrien De Maeyer
- Jolien D'aes
- Sarah Van Beneden
- Soraya De Carvalho França
- Ilse Delaere
- "Special Research Fund" (BOF) Ghent University