Effects of the bacteria *Pseudomonas fluorescens* on the mycorrhization between *Cistus ladanifer* and *Boletus edulis*

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Introduction

Drastic decrease productions

Ever-increasing demand

Collected only from wild

CONTROLLED PRODUCTIONS

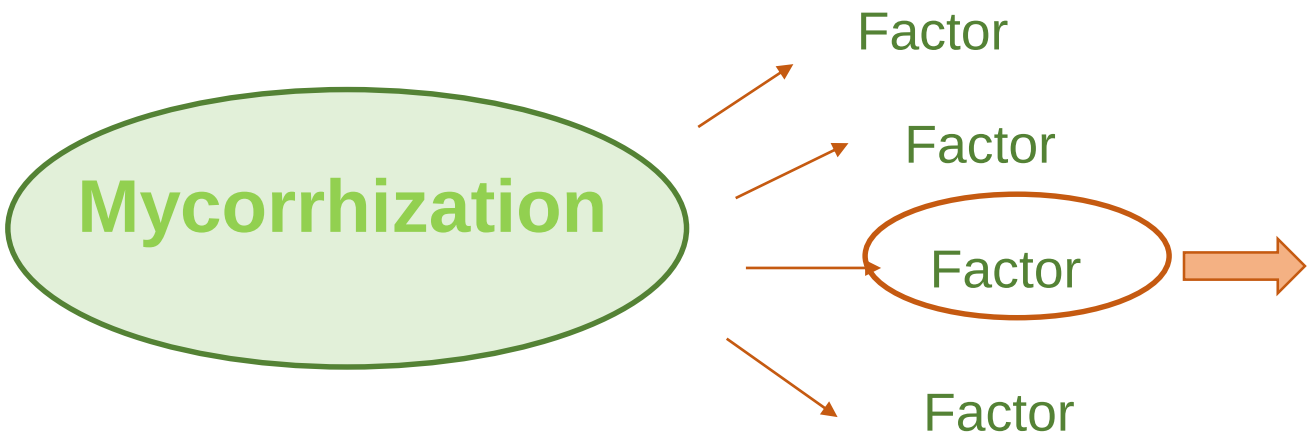
Introduction

3 YEARS OLD! *Cistus ladanifer* plants

Large-scale plant production → MICROPROPAGATION

- ✓ Fast method
- ✓ Selecting plant material
- ✓ Higher number of mycorrhized plants

Introduction



Microorganisms of the rhizosphere

Bacteria enhance mycorrhizal symbiosis → MHB *Pseudomonas fluorescens*

Positive results → NEW EXPECTATIVES TOWARDS DOMESTICATION!!

Objectives

To optimize a protocol for the mycorrhizal synthesis of *Boletus edulis* with *Cistus ladanifer* vitroplants by assessing the effects of coinoculation with *Pseudomonas fluorescens*

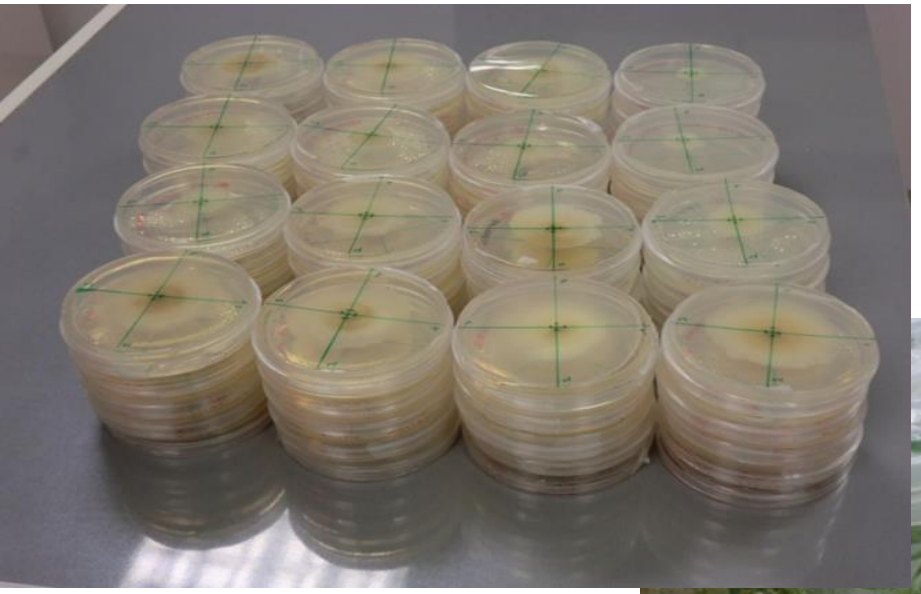
Specifics objectives:

1. To assess the influence of ***P. fluorescens*** on the level of mycorrhization

2. To assess the influence of **the mycelium culture time** on the level of mycorrhization.

Materials and Methods

FUNGAL INOCULUM



PLANT MATERIALS

BACTERIAL INOCULUM

Materials and Methods

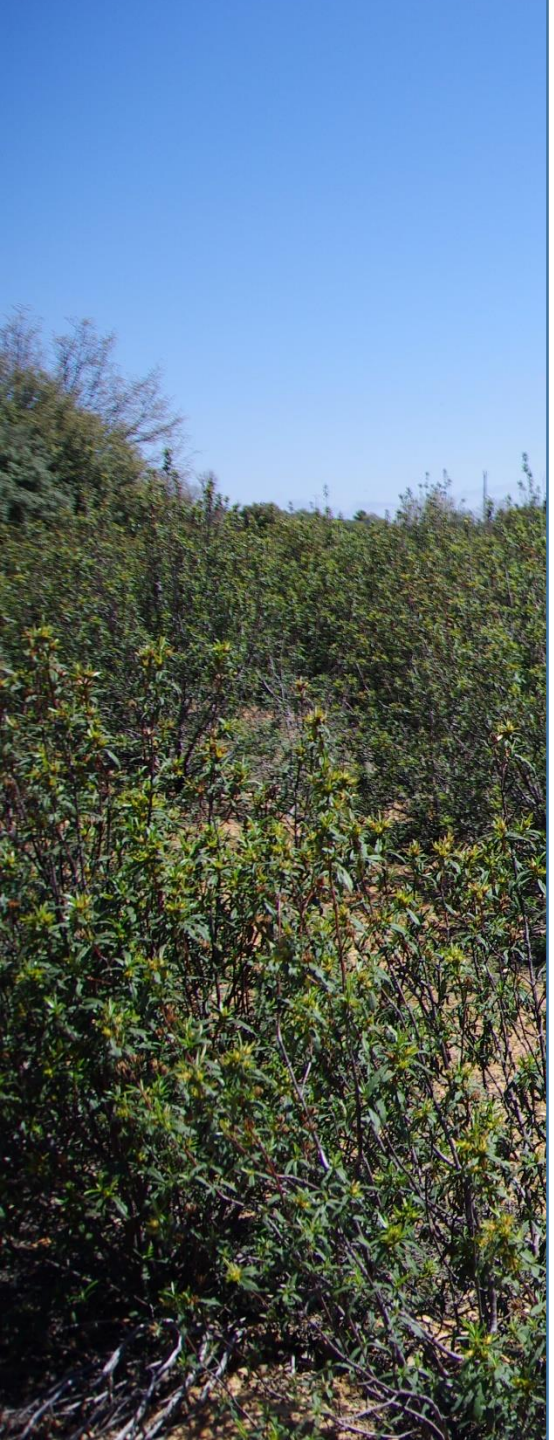
FUNGAL INOCULUM

- Collection and isolation of *B. edulis* sporocarps
- Growing in MMN nutritive medium
- Inoculation in solid expanding substrate
- Inoculated substrate grown: 2, 3 or 4 months

Materials and Methods

BACTERIAL INOCULUM

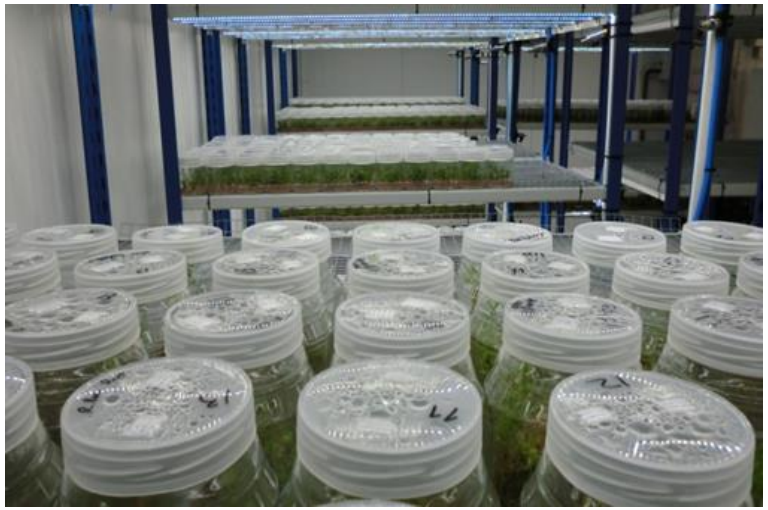
- *Pseudomonas fluorescens* strain supplied by CECT (Valencia University)
- Grown in malt-glucose nutritive medium
- Inocula re-suspension to 5.10^{8} bacteria/ml



Materials and Métodos

PLANT MATERIALS

- Collected from shrubs hosting *B. edulis*
- Propagation on MS basal medium
- Rooting into MS+ 0.49 mg/l IBA
- Plants grown 2 months, 25±1ºC, 16h photoperiod



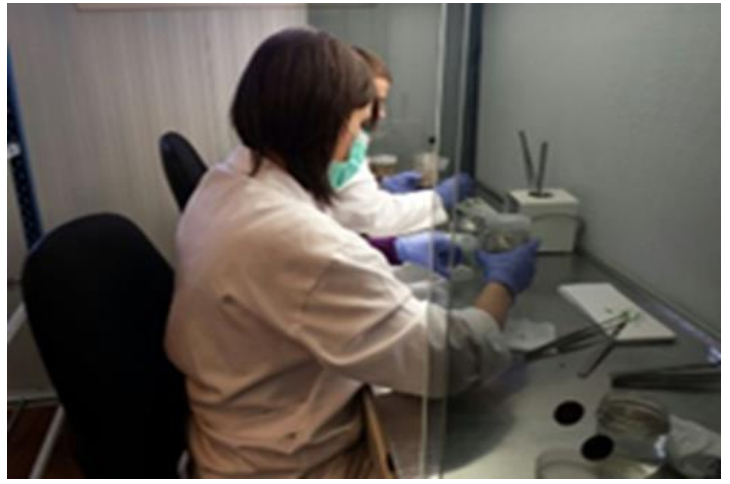
Materials and Methods

MYCORRHIZAL AND BACTERIAL INOCULATION

- *C. ladanifer* vitroplants
  - → *B. edulis* inoculated pots
  - → Control pots

- Half of the plants inoculated with *P. fluorescens* (5.10^{8} bacteria/plant)

- Plants grown in growth chamber 5 months, 25±1ºC, 16h photoperiod



Materials and Methods

EXPERIMENTAL DESIGN

- **Three inoculation types:**
  - **1. Inoculation with *B. edulis***
  - **2. Inoculation with *B. edulis* + *P. fluorescens***
  - **3. Control (non-inoculated)**
- **Three mycelium culture time (2, 3 or 4 months)**
- **18 pots/treatment, 4 plants/pot: 504 plants tested**

Materials and Methods

MYCORRHIZAL COLONIZATION VERIFICATION

- ✓ Morphological identification
  - Stereomicroscope
- ✓ Molecular analysis
- ✓ Counting of root tips (level of mycorrhization)
  - Total tips
  - Mycorrhized tips

Results

MYCORRHIZAL SYNTHESIS

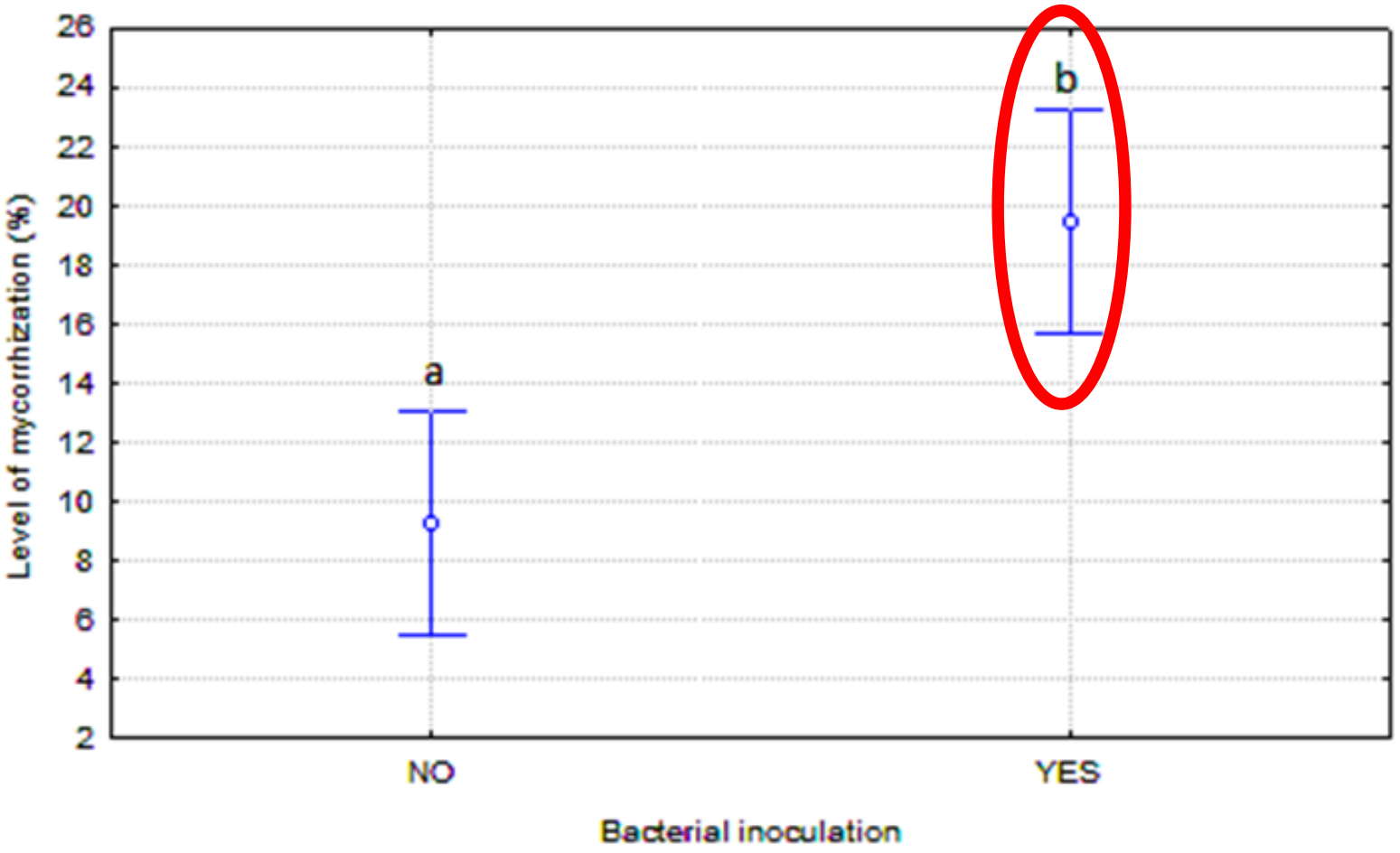
Inoculation type	Mycorrhization
B. edulis	✓
B. edulis + *P. fluorescens*	✓
Control plants	✗

Mycorrhizal synthesis SUCCESSFUL

Results

LEVEL OF MYCORRHIZATION WITHIN THE MYCORRHIZED PLANTS

- Bacteria coinoculation



Results

LEVEL OF MYCORRHIZATION WITHIN THE MYCORRHIZED PLANTS

BACTERIA

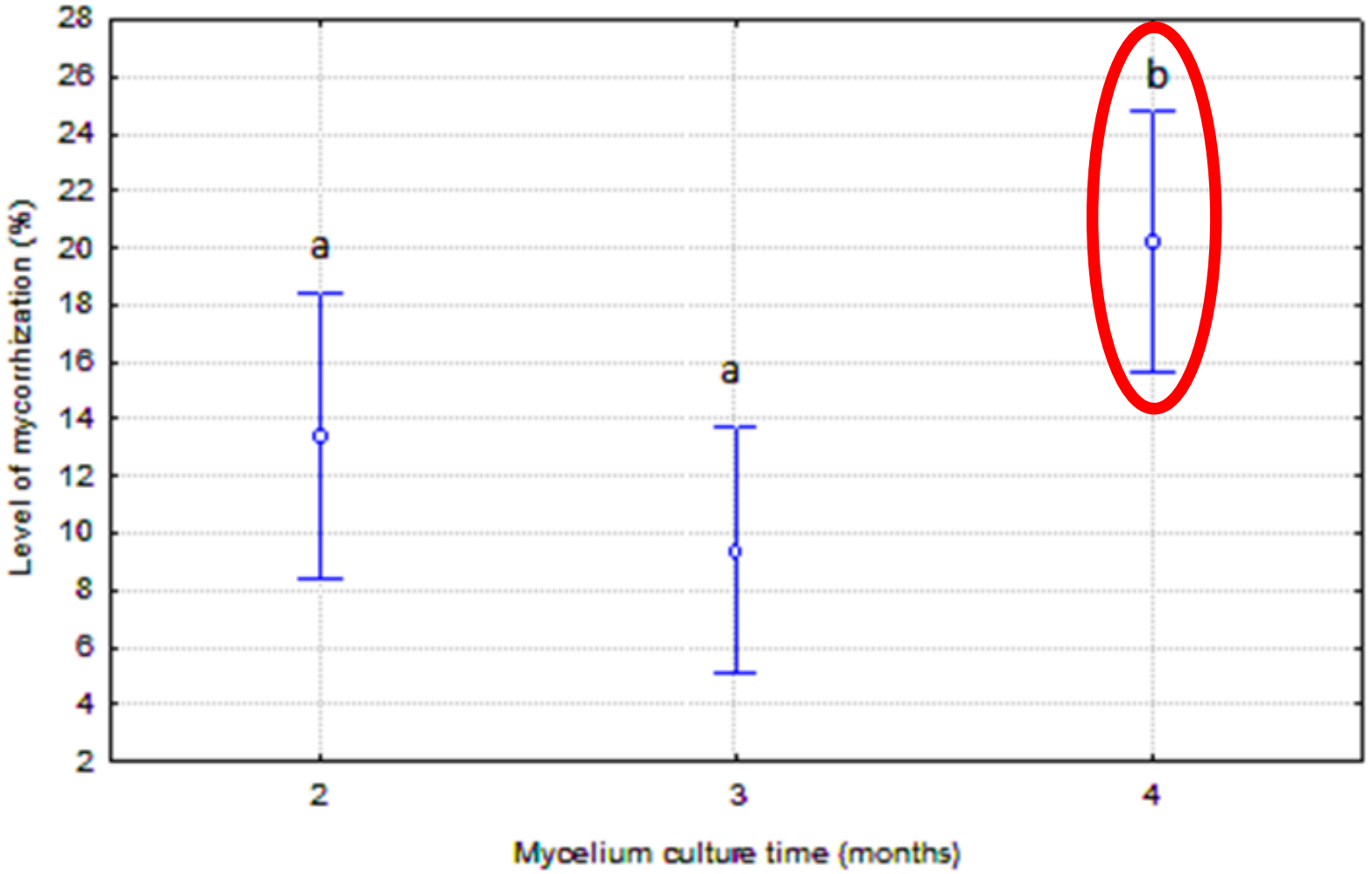
Mycelium culture time	Inoculation	
	Be	BexPf
2 months	6.98±2.73aA	18.55±3.48bAB
3 months	6.32±2,83aA	11.91±2.63aA
4months	14.28±3.11aA	23.49±2.63bB

TIME

Results

LEVEL OF MYCORRHIZATION WITHIN THE MYCORRHIZED PLANTS

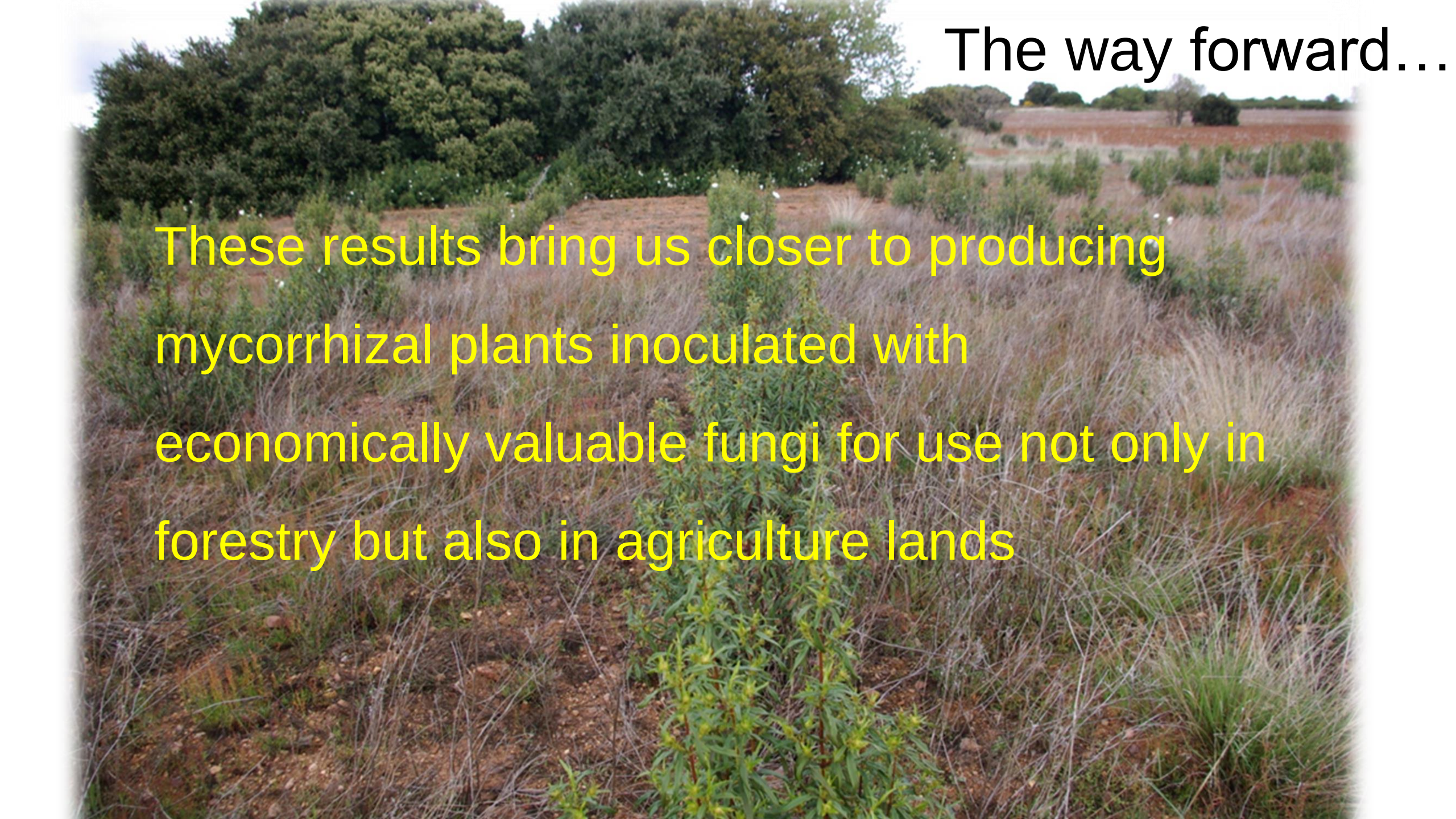
- Mycelium culture time



Conclusions

- **Mycorrhizal synthesis** between *C. ladanifer* and *B. edulis* was achieved **successfully**
- The results obtained confirmed the **beneficial effects of *P. fluorescens*** in enhancing the level of mycorrhization compared to inoculation with *B. edulis* alone.
- **Longer mycelium culture** times also improved the level of mycorrhization.
- The use of *C. ladanifer* **vitroplants** may allow **more efficient** production of mycorrhized plants compared to use of inoculated seedlings.

The way forward…



These results bring us closer to producing mycorrhizal plants inoculated with economically valuable fungi for use not only in forestry but also in agriculture lands

Reference:

Mycorrhiza (2016) 26:161–168
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ORIGINAL ARTICLE

Mycorrhization between *Cistus ladanifer* L. and *Boletus edulis* Bull is enhanced by the mycorrhiza helper bacteria *Pseudomonas fluorescens* Migula

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Thanks for your attention!

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