Biotechnological application of fungi as biological control agents against olive pests and diseases

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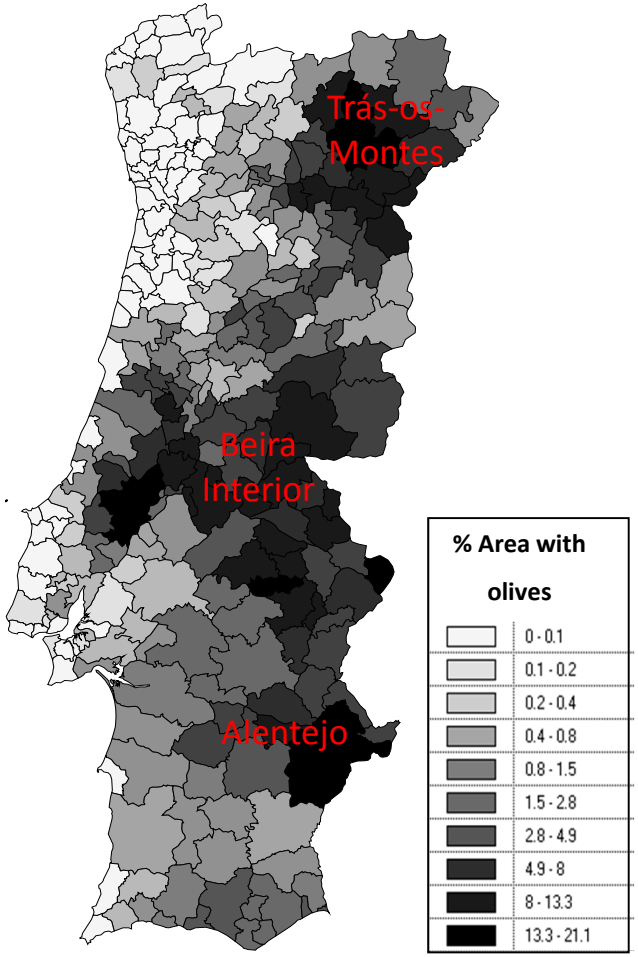
Laboratory AgroBioTechnology / Escola Superior Agrária de Bragança

Olive culture

Olive tree is an important crop in the Mediterranean basin, including Portugal.



Talhinhas et al. (2010) Plant Pathology 60: 483–495

- The area occupied by the olive tree is 360 000 ha

- Portugal is the 8th largest producer of olive oil

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Olive culture constrains

Key insect pest

Olive moth

Olive fruit fly

Main diseases

Olive anthracnose

Verticillium wilt

Reducing crop production by 30%

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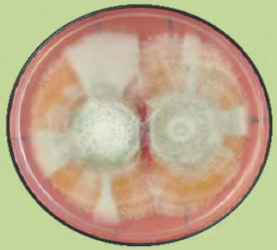
Main olive diseases

Anthracnose

Colletotrichum spp.



C. acutatum (97%)

C. gloeosporioides (3%)

Olive anthracnose

Verticillium wilt

Verticillium

Verticillium dahliae

Verticillium wilt

Key olive pest

Prays oleae Bern

Olive moth

- **Three annual generations:**

October to March	April May June	July August September
Phyllophagous	Antophagous	Carpophagous

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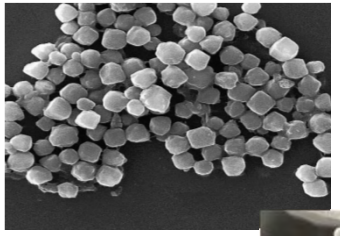
Biological control

Use of live ORGANISMS or their PRODUCTS → Aiming at eliminating or reducing PEST / DISEASES losses

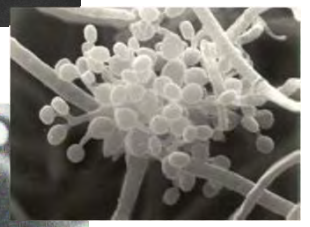
PARASITOIDS

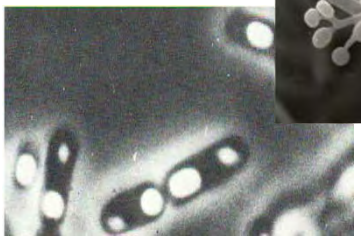
PREDATORS

BIOLOGICAL CONTROL



VIRUS





FUNGI

BACTERIAS

NEMATODES

MICROBIOLOGICAL CONTROL

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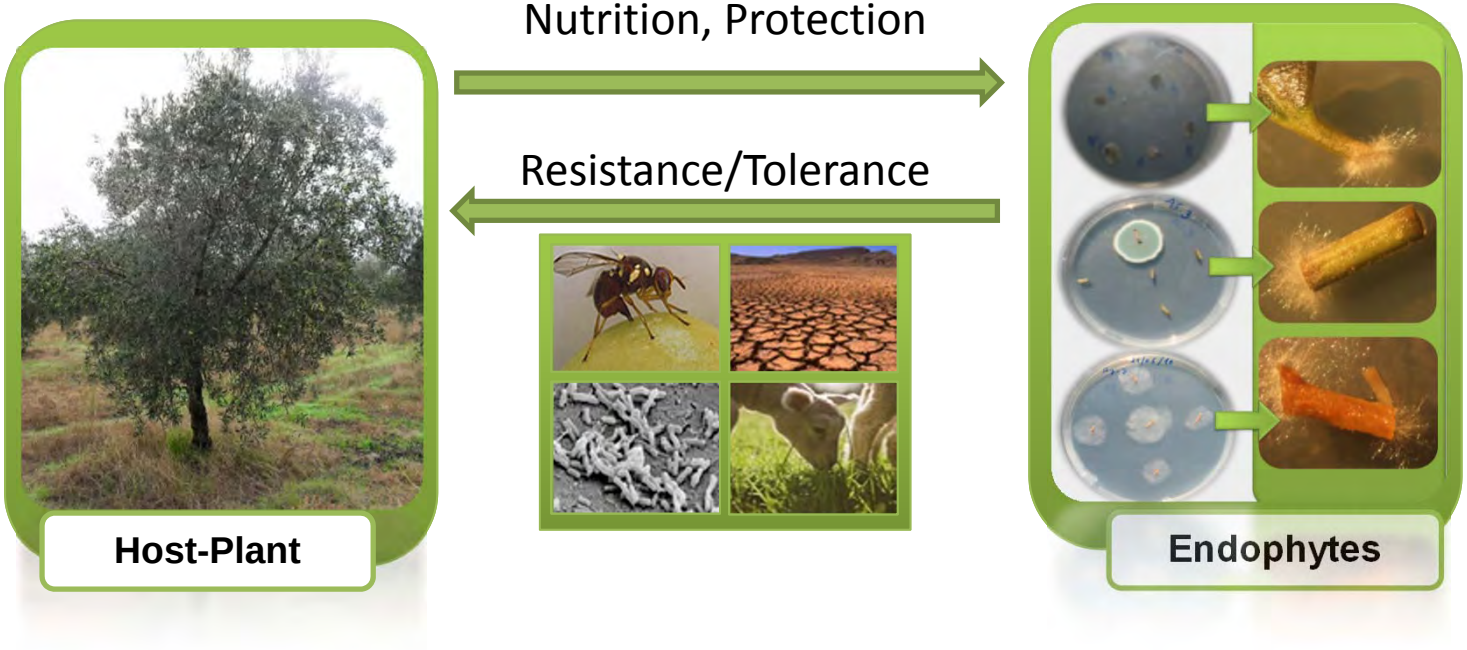
Biological control of diseases

Fungal Endophytes

Fungal endophytes are a diversified group of microorganisms that live asymptomatically within most plant tissues.

Endophyte-host plant interactions



Biological control of diseases

Some successful cases of endophytes fungal application in the biological control

Mycol. Res. **107** (3): 310–316 (March 2003). © The British Mycological Society
DOI: 10.1017/S0953756203007512 *Printed in the United Kingdom.*

Some endophytic fungi reduce the density of pustules of *Puccinia recondita* f. sp. *tritici* in wheat

Juliet DINGLE and Peter A. MCGEE*

Mycol. Res. **109** (5): 610–618 (May 2005). © The British Mycological Society
doi:10.1017/S0953756205002820 *Printed in the United Kingdom.*

A protective endophyte of maize: *Acremonium zeae* antibiotics inhibitory to *Aspergillus flavus* and *Fusarium verticillioides*[1]

Donald T. WICKLOW[1], Shoshannah ROTH[2], Stephen T. DEYRUP[2] and James B. GLOER[2]

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Available online at www.sciencedirect.com
ScienceDirect
Biological Control 46 (2008) 4–14

Biological Control
www.elsevier.com/locate/ybcon

Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens

Luis C. Mejía [a,b], Enith I. Rojas [a], Zuleyka Maynard [a], Sunshine Van Bael [a], A. Elizabeth Arnold [c], Prakash Hebbar [d], Gary J. Samuels [e], Nancy Robbins [a], Edward Allen Herre [a,*]

Up to date, studies on fungal endophytes of olive tree are still lacking and their involvement on plant disease resistance was never studied…

Biological control of pests

Entomopathogenic fungi

- About 700 insect hosts
- Present a cosmopolitan distribution
- Proposed as biocontrol agents over 130 years ago
- Used in the formulation of bio-insecticides
- Several advantages regarding chemical insecticides

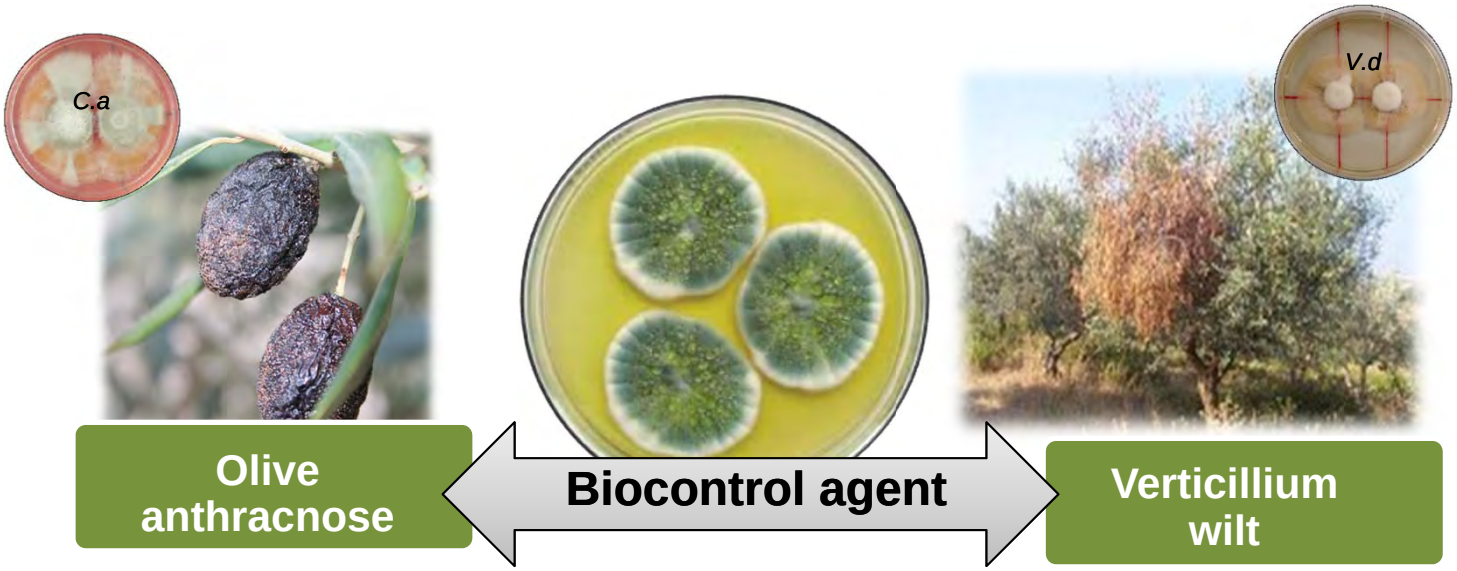
Up to date, studies on entomopathogenic fungi associated with *Prays oleae* are still lacking and their involvement on the biological control of this pests was never studied…

Biological control of diseases

Project PTDC/AGR-PRO/4354/2012

Fátima Martins
IPB/ESA

- Assess the diversity of fungal endophytes in 3 olive cultivars with different susceptibilities to *C. acutatum and V. dhaliae*:

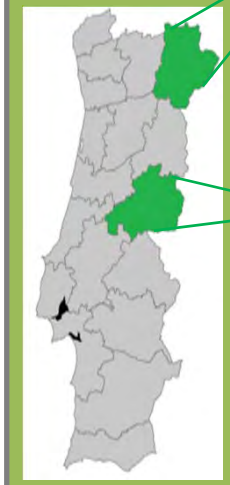


The differences on the fungal community found in *cvs*. are expected to be somehow correlated with plant susceptibility to diseases

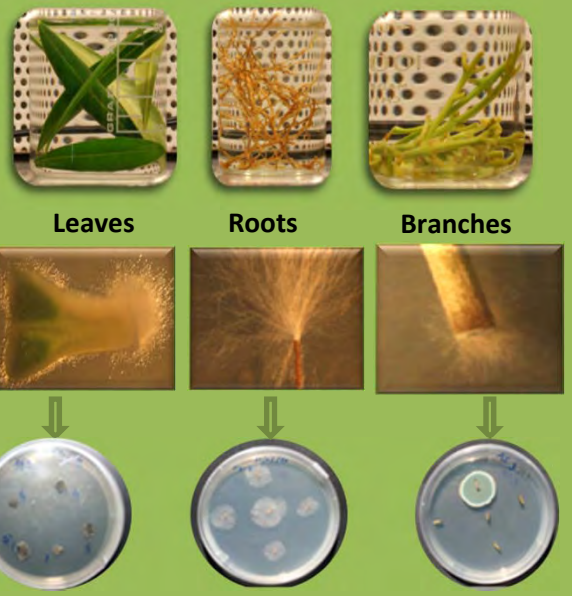
- Select endophytes with antagonist potential against both diseases

Procedure

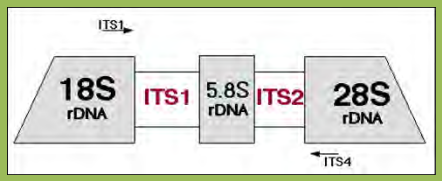
Collection of plant material

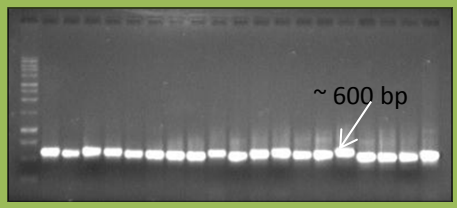


Isolation of fungal endophytes

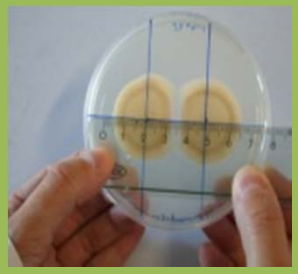


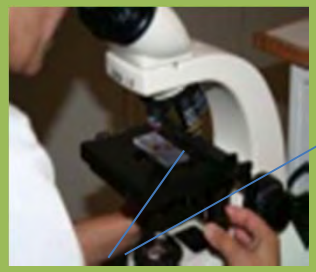
Molecular identification of fungal isolates by rDNA sequencing

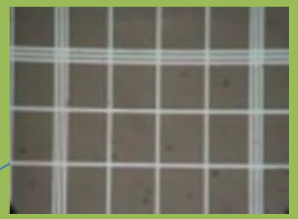




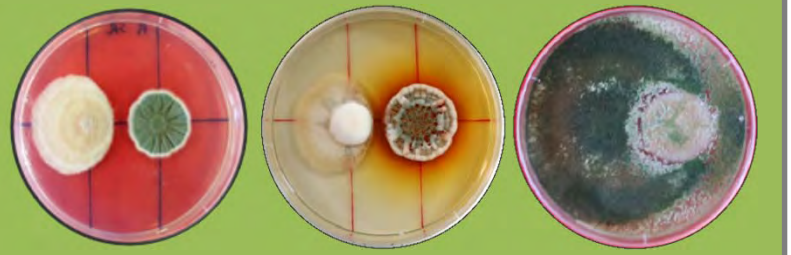
Macro and microscopic characterization of dual cultures







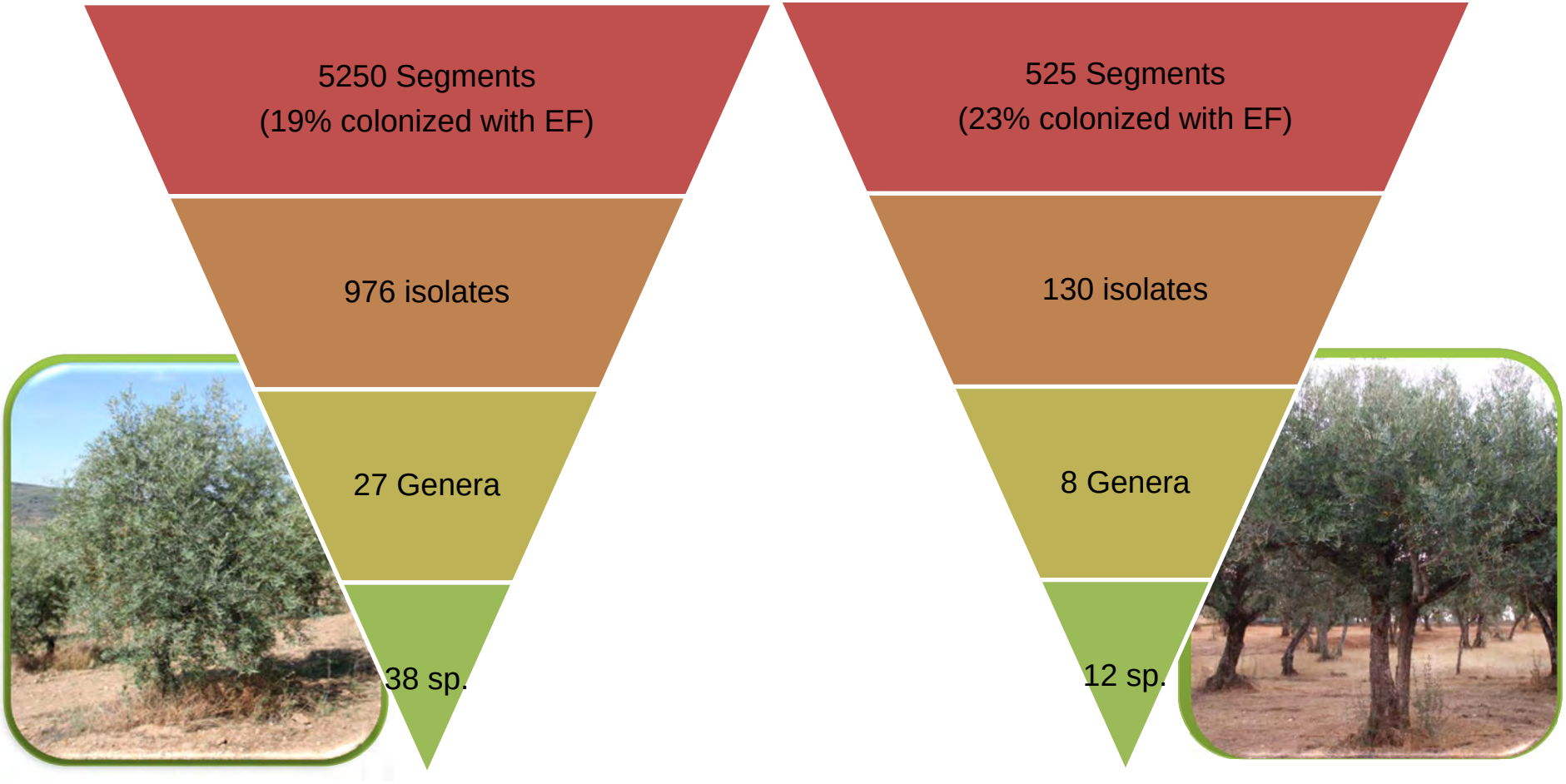
Establishment of dual culture with *C. acutatum and V. dahliae*



Results

Fungal endophyte community

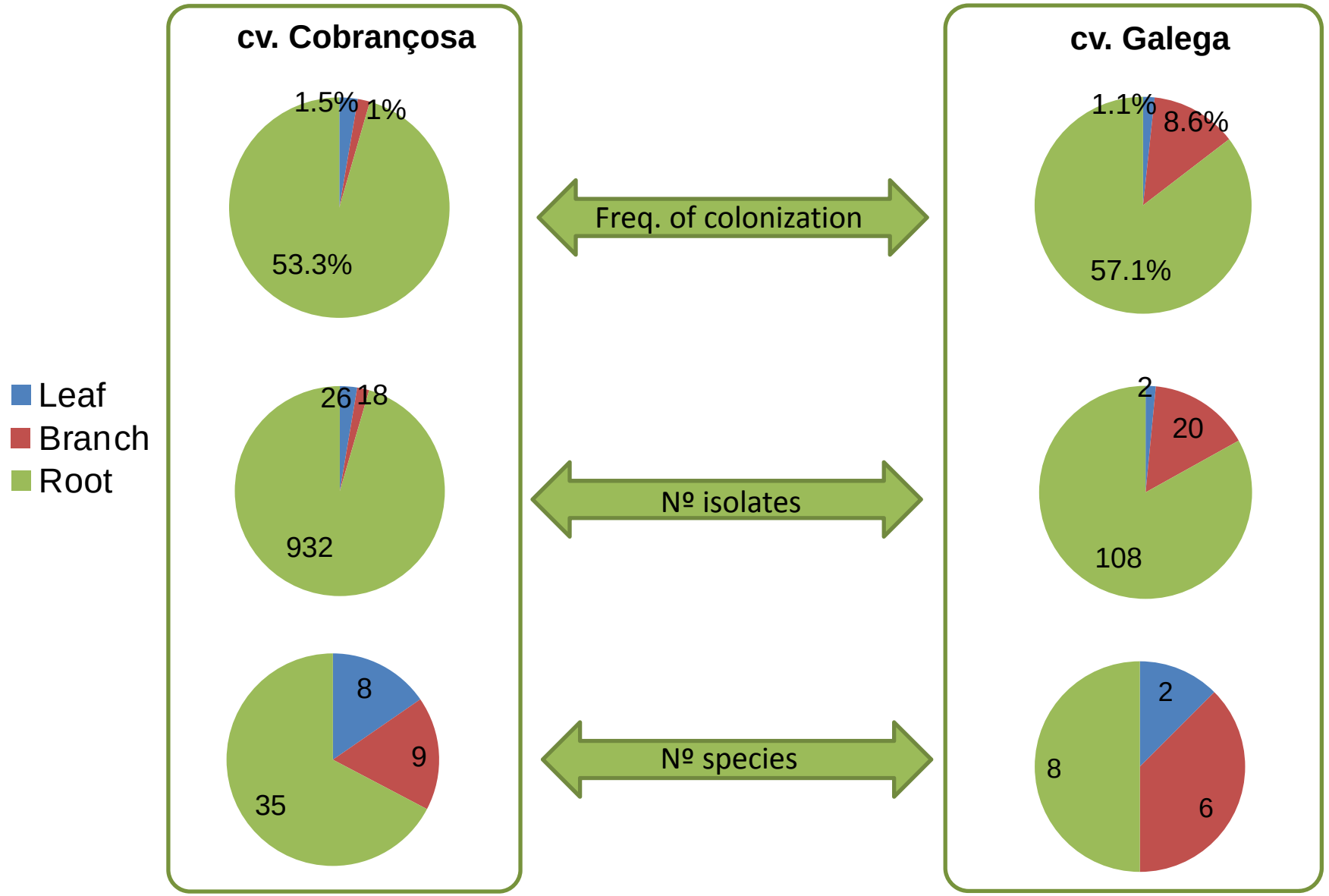
cv. Cobrançosa



cv. Galega

Results

Fungal endophyte community



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Fungal endophyte community

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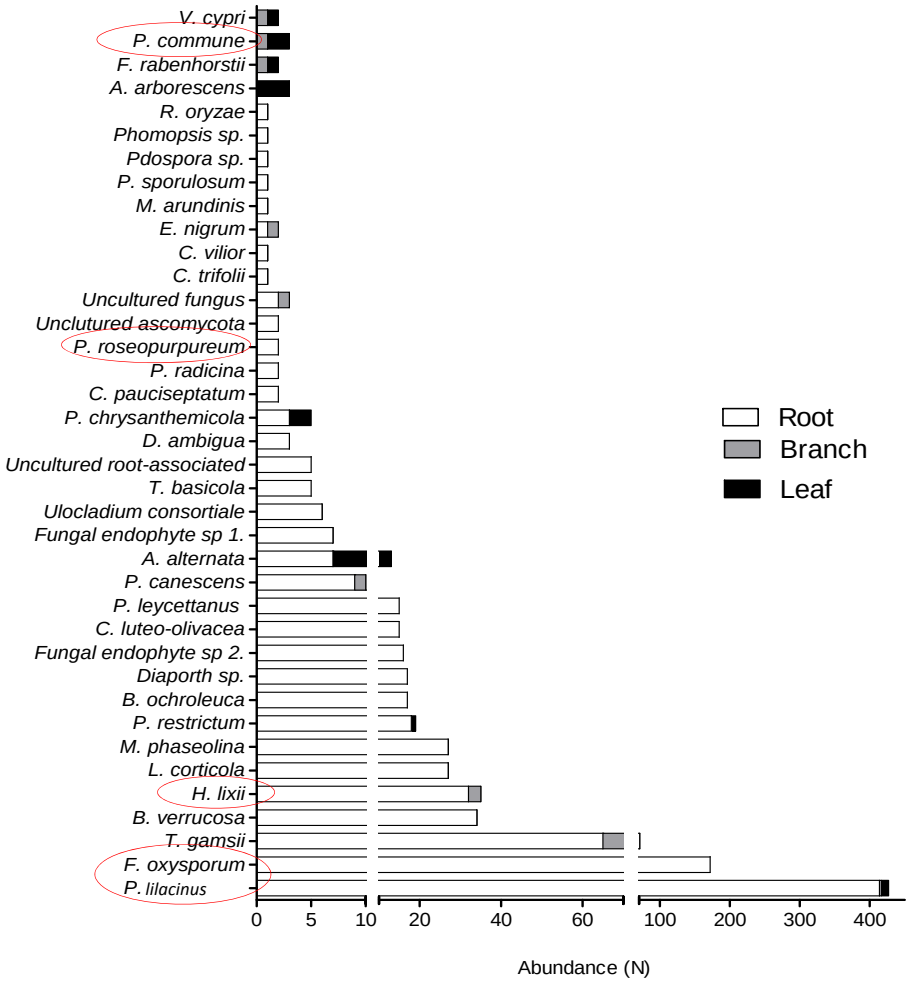
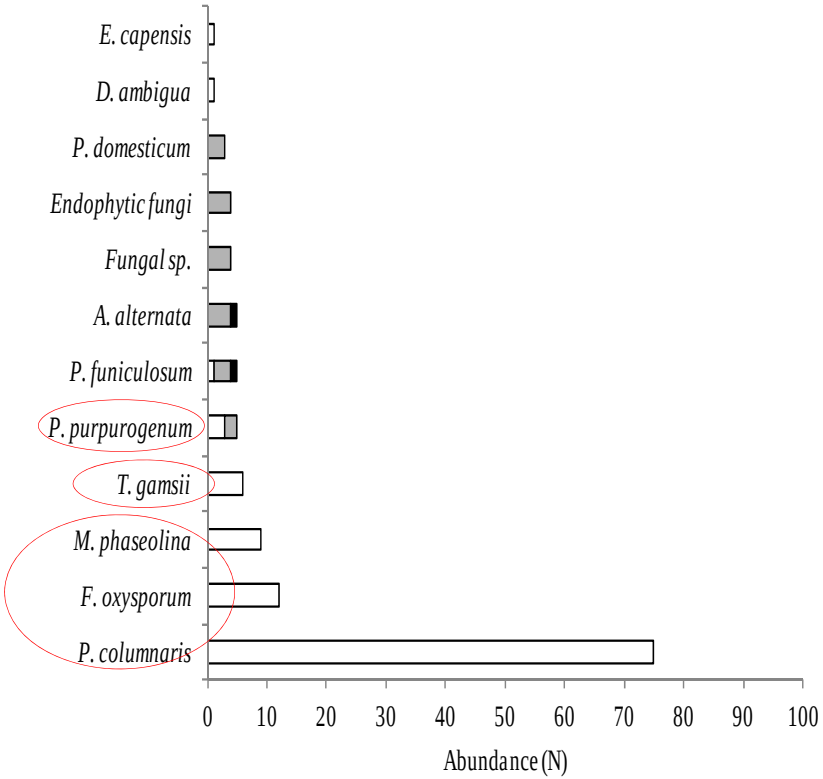


cv. Galega



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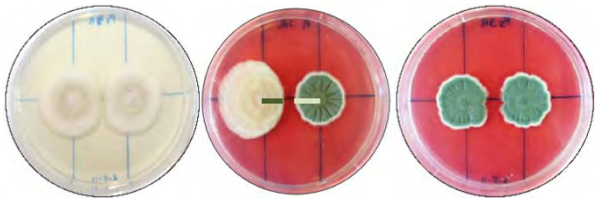
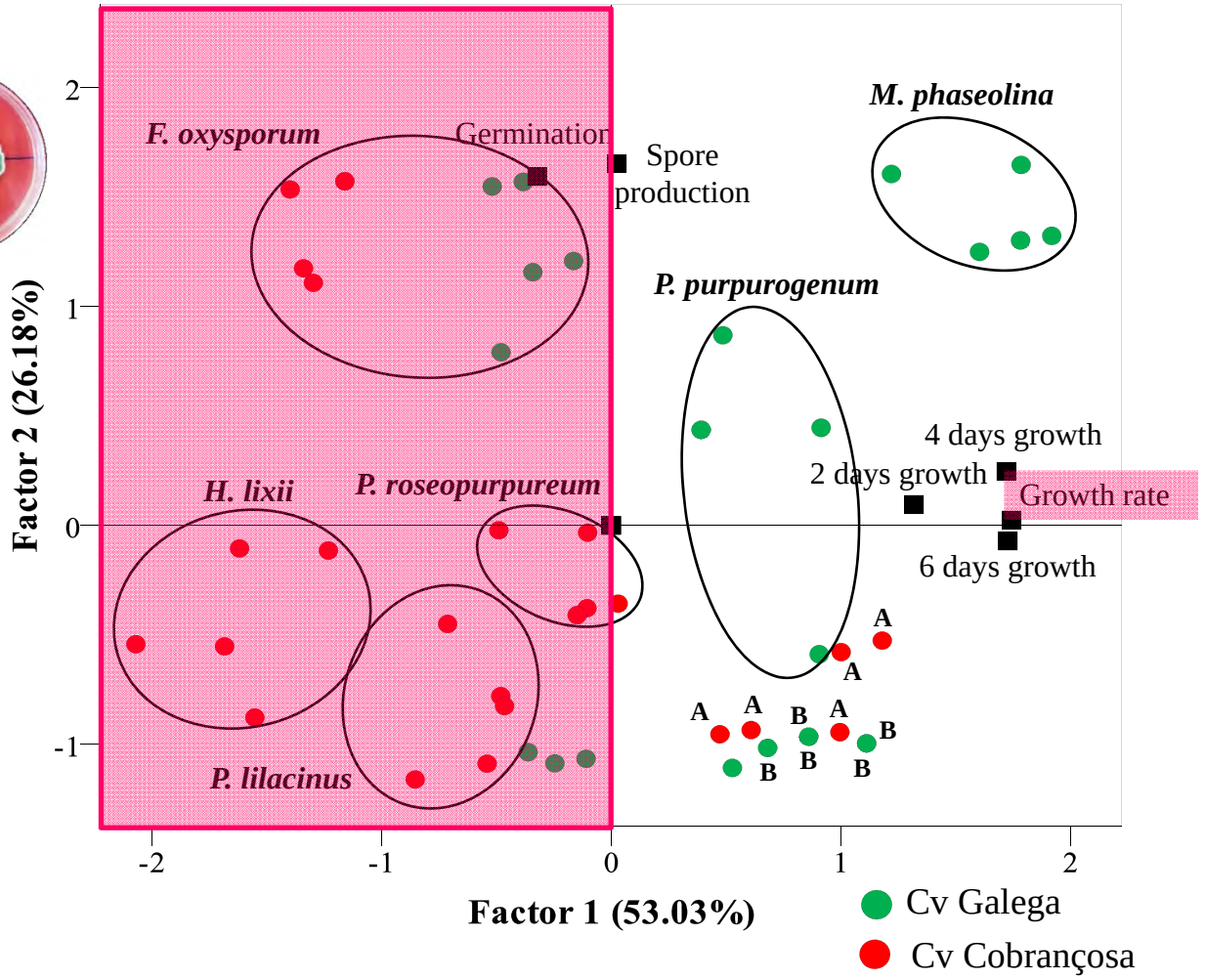
Effect of endophytic fungi isolated from *cvs.* Cobrançosa and Galega on *C. acutatum* growth, sporulation and germination

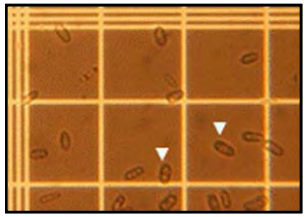


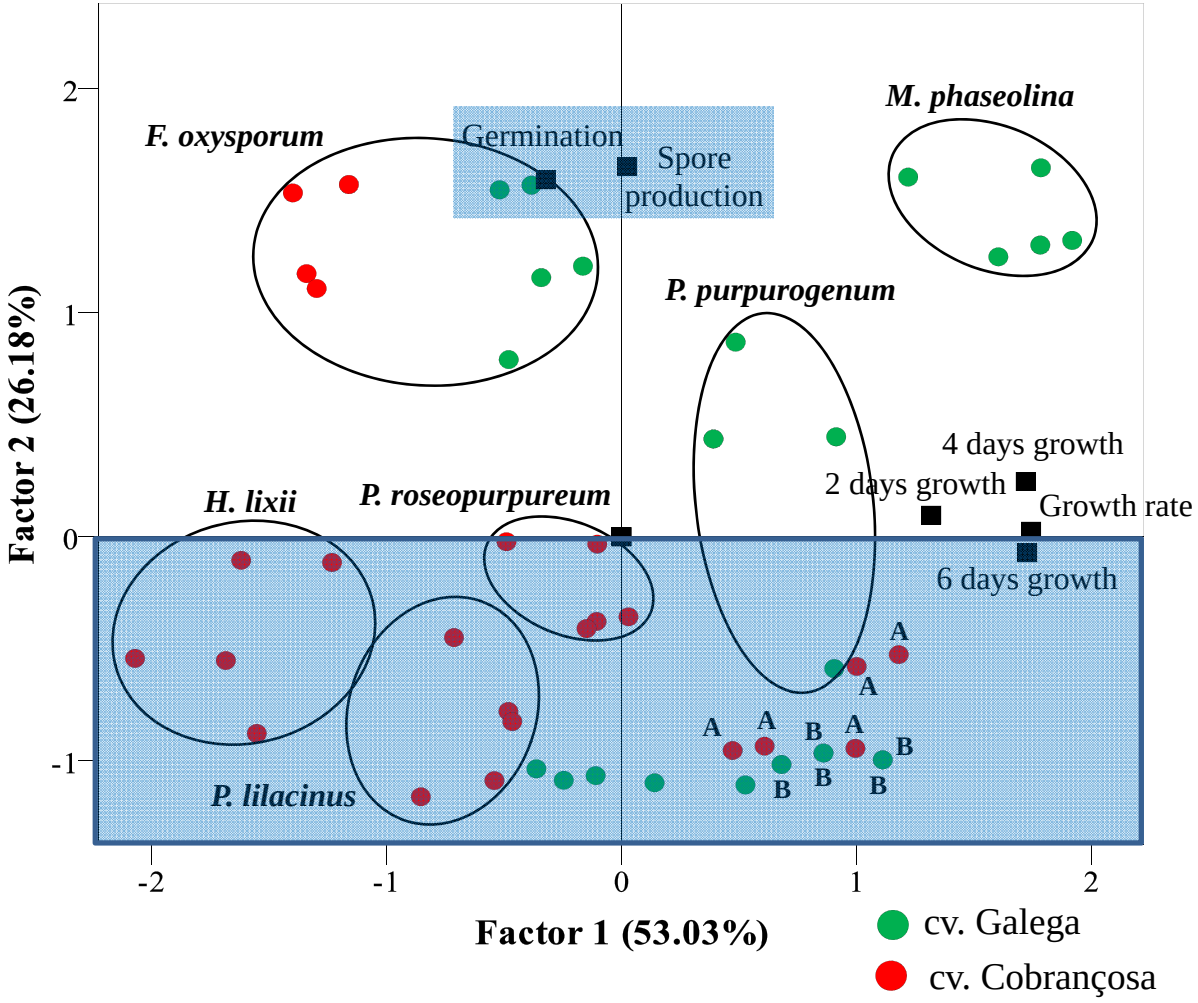


The two principal factors explain 79.21% of the total variance of data. A – *P. commune*; B – *T. gamsii*.

Results

Effect of endophytic fungi isolated from *cvs.* Cobrançosa and Galega on *C. acutatum* growth, **sporulation** and **germination**





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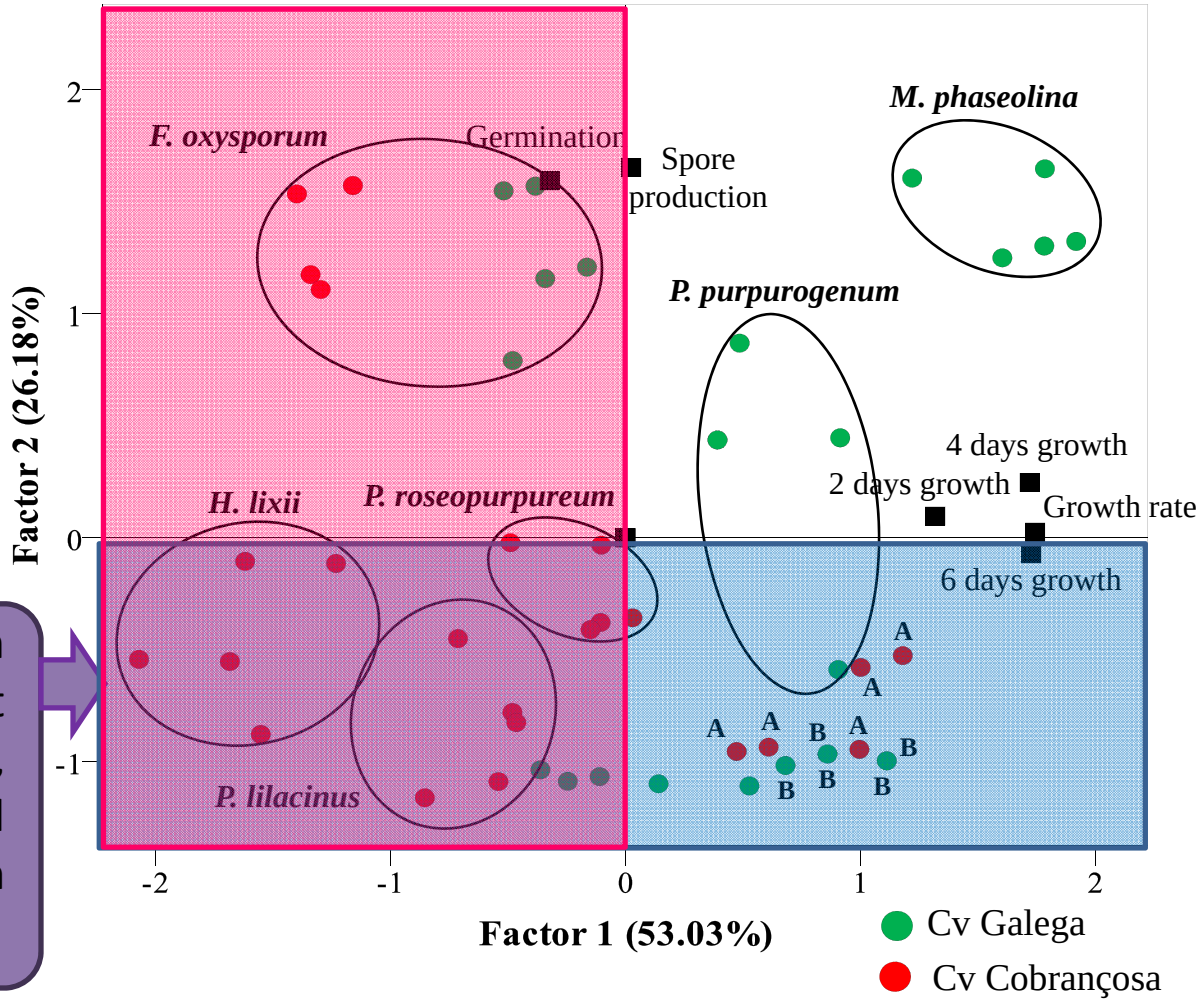


The EF species with greatest inhibitory effect on *C. acutatum* growth, sporulation and germination were from cv Cobrançosa

The two principal factors explain 79.21% of the total variance of data. A – *P. commune*; B – *T. gamsii*.

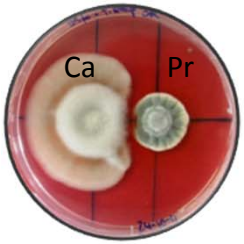
Results

cv. Cobrançosa

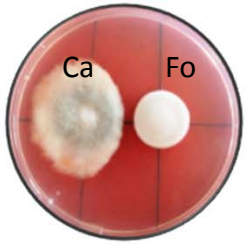
Co-antagonism

Antagonism

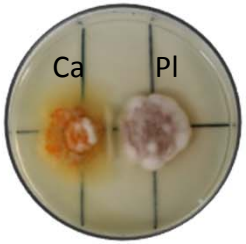
Macroscopic



(-/-)



(-/-)



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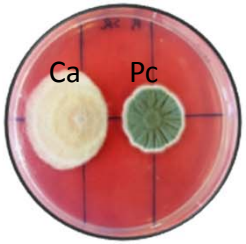
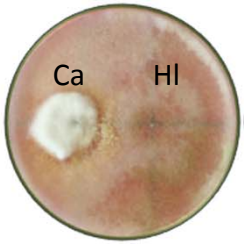
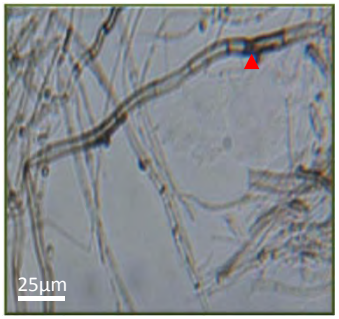


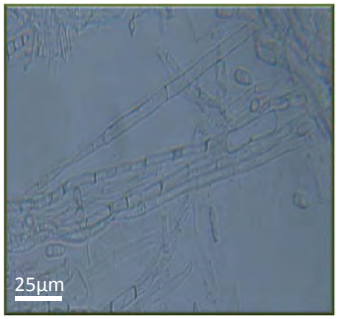
(-/0)



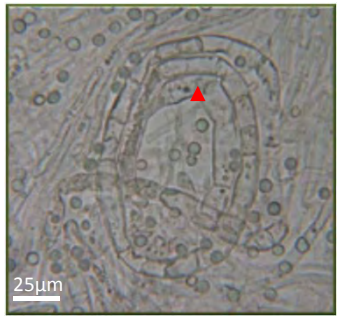
(-/0)

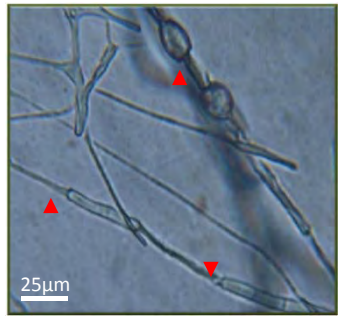
Microscopic
(at interaction zone)

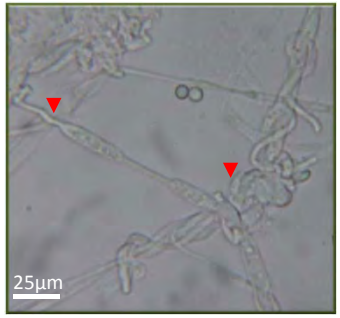


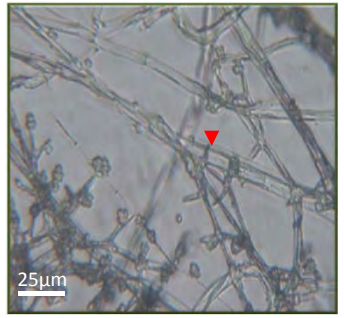


No changes







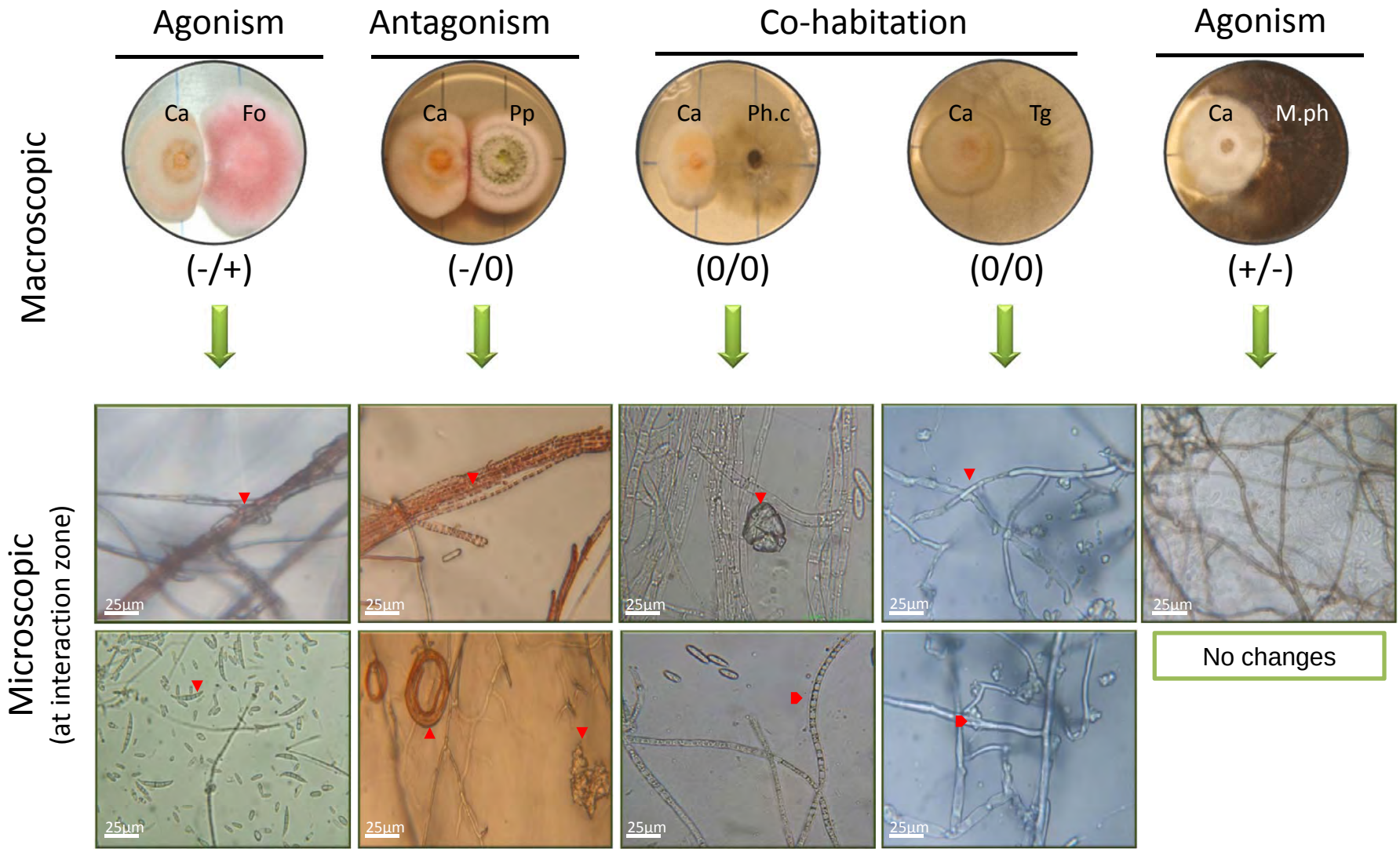


Ca - Colletotrichum acutatum
Pr - Penicillium roseopurpureum
Fo - Fusarium oxysporum
Pl - Paecilomyces lilacinus
Pc - Penicillium commune
Hl - Hypocrea lixii

The terminology used to described the interspecific interaction are based on Tuininga (2005) *The fungal community*, Taylor and Francis.

Results

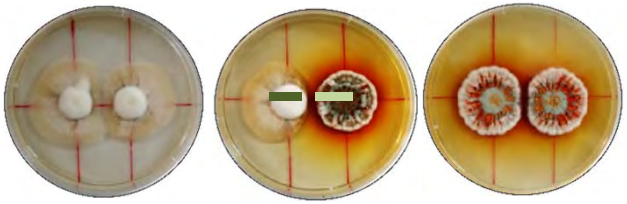
cv. Galega

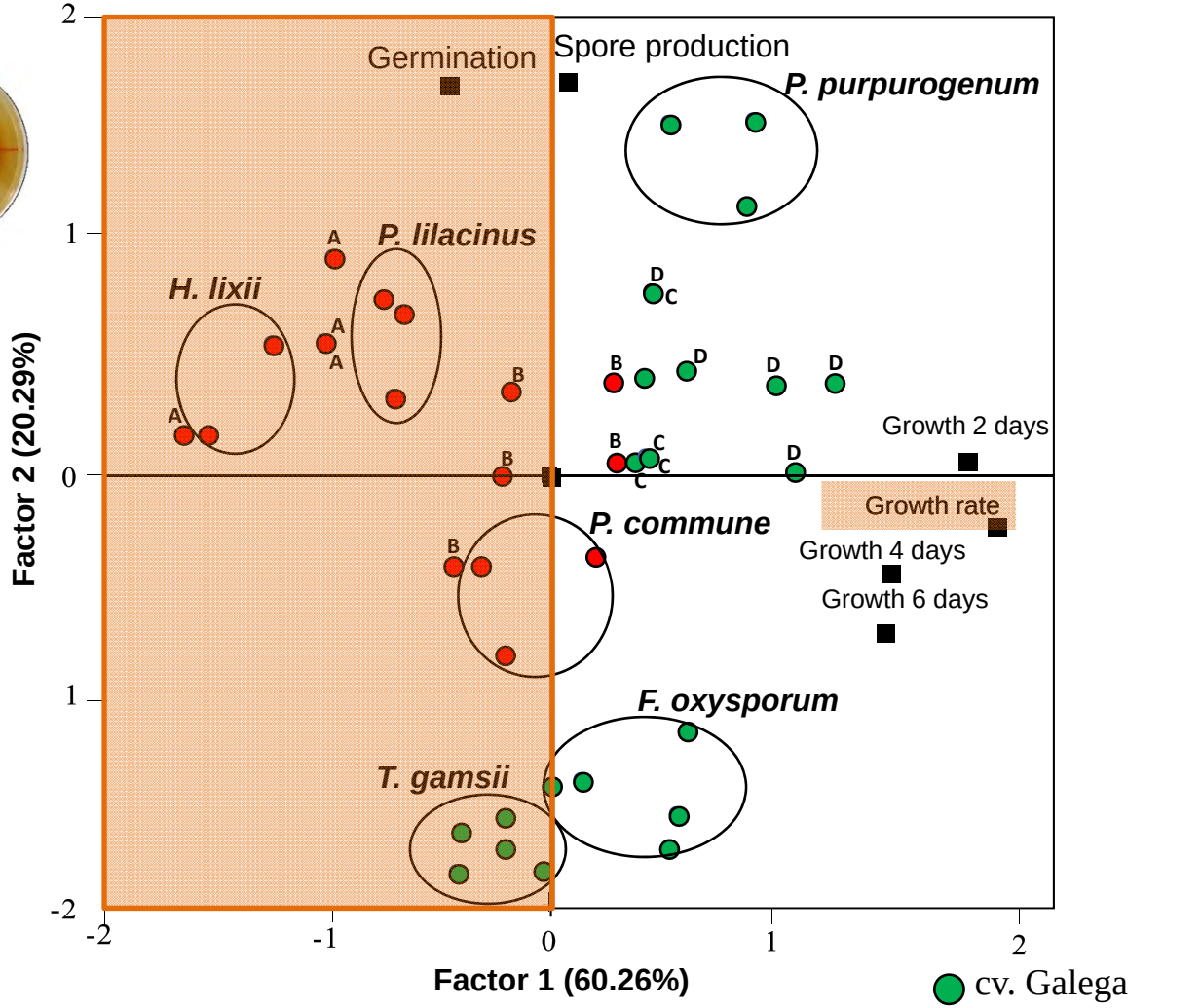


Ca - Colletotrichum acutatum; Fo - Fusarium oxysporum; Pp - Penicillium purpurogenum; Ph.c - Phomopsis columnaris; Tg - Trichoderma gamsii; M.ph - Macrophomina phaseolina.

Results

Effect of endophytic fungi isolated from *cvs.* Cobrançosa and Galega on *V. dahliae* growth, sporulation and germination





The two principal factors explain 80.55% of the total variance of data.

A- *P. roseopurpureum*; B- *F. oxysporum*; C- *M. phaseolina*; D- *Ph.columnaris*.

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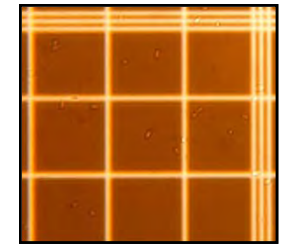

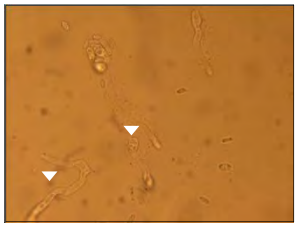

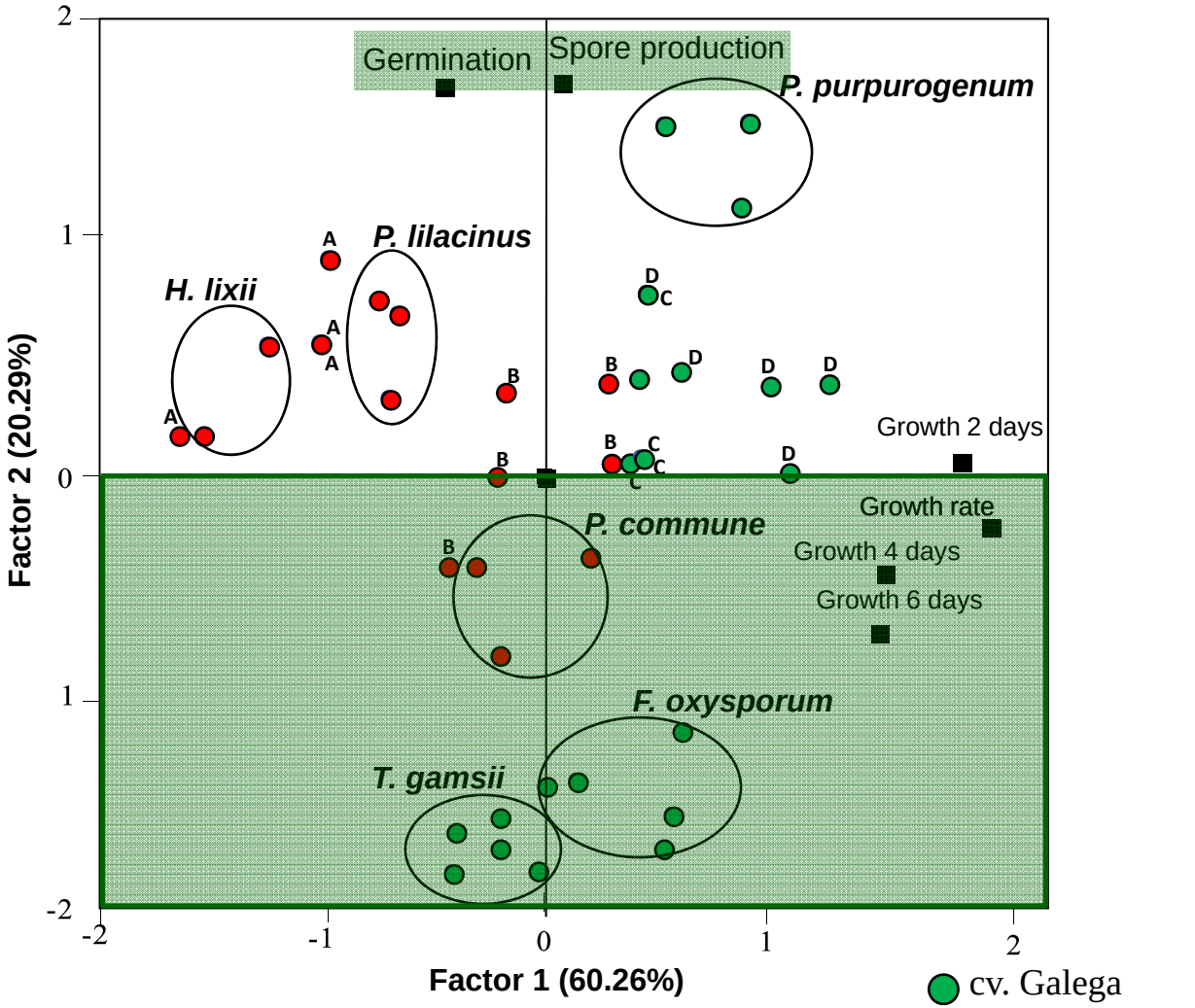

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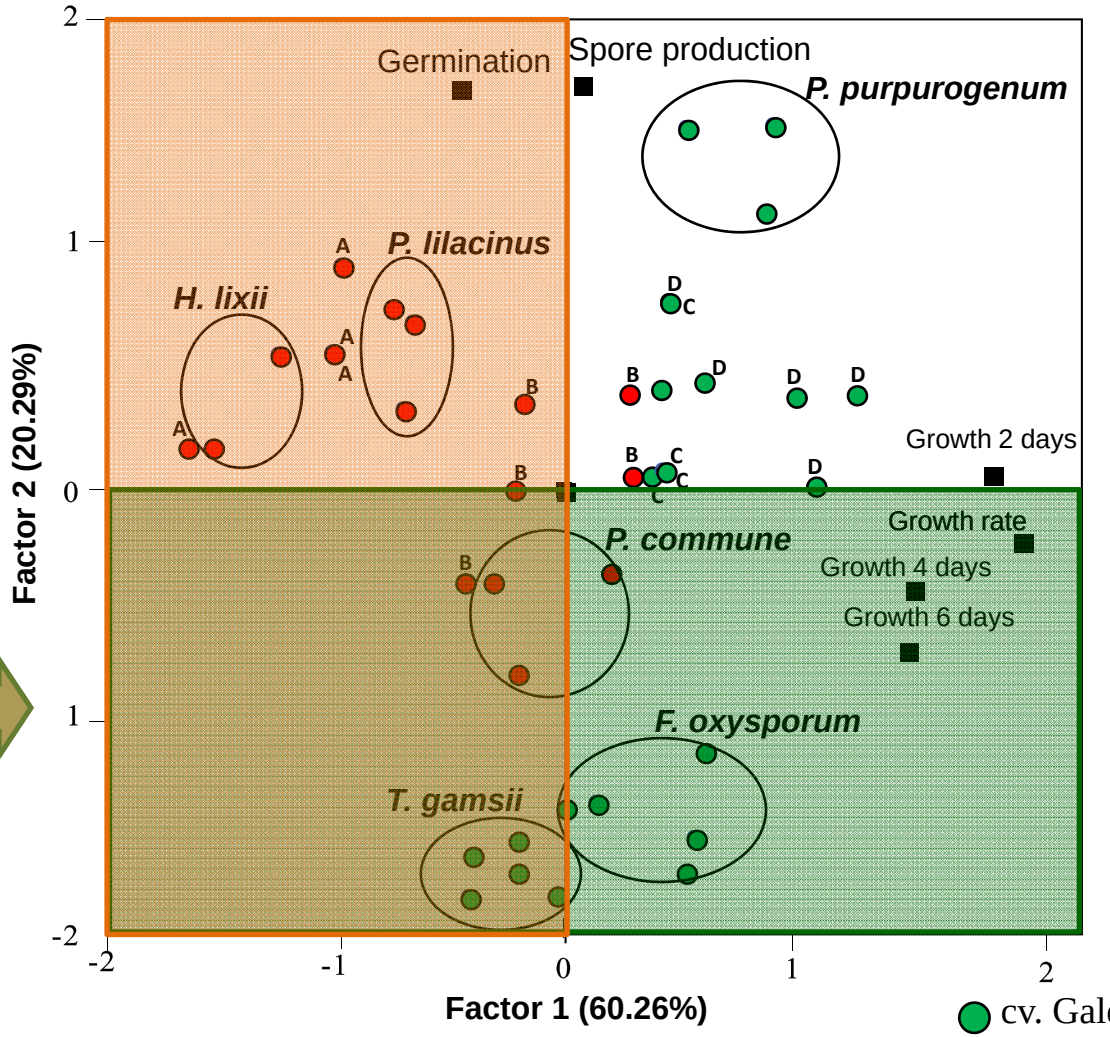


The EF species with greatest inhibitory effect on *V. dahliae* growth, sporulation and germination were from cv Galega

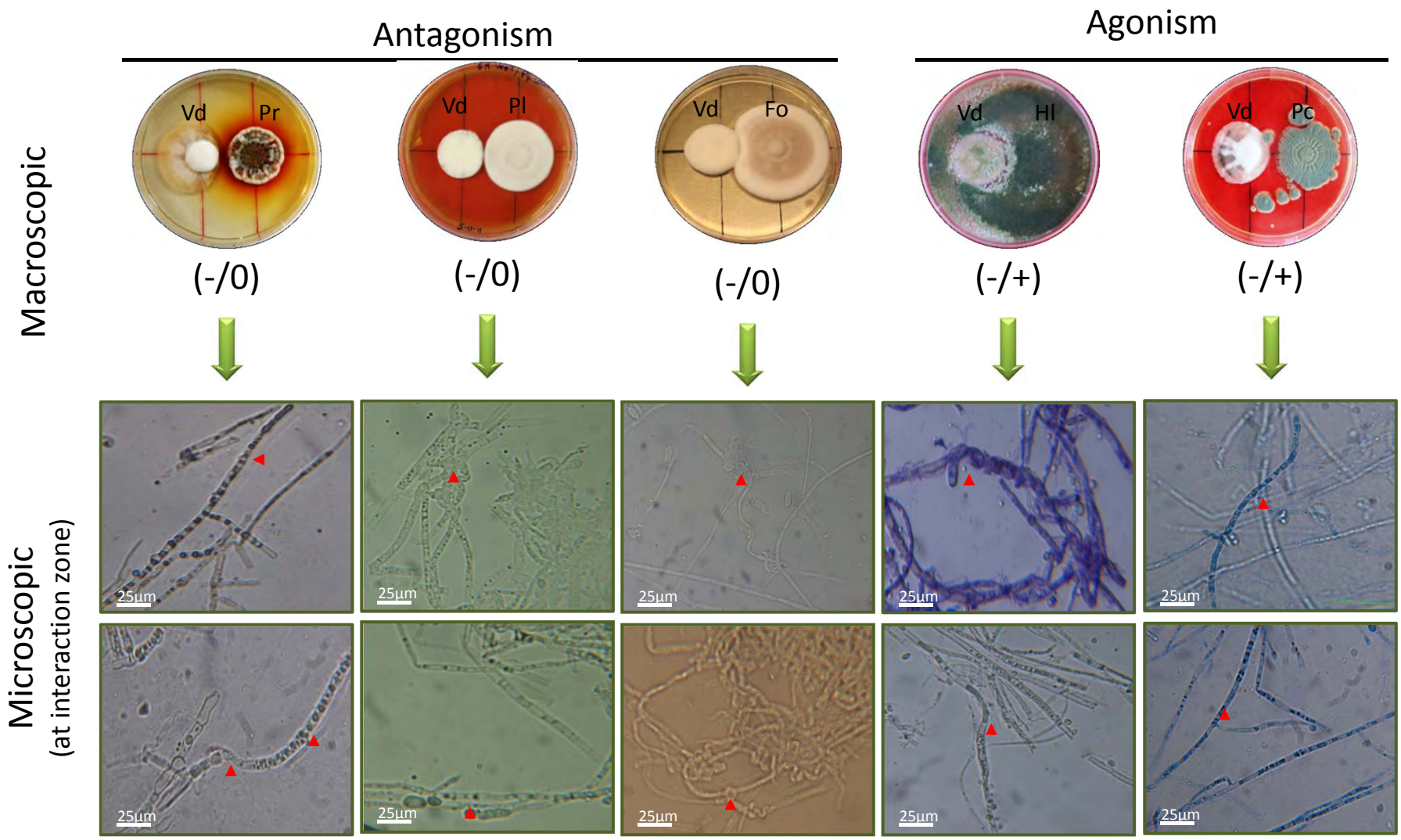
cv. Galega
cv. Cobrançosa

The two principal factors explain 80.55% of the total variance of data.

A- *P. roseopurpureum*; B- *F. oxysporum*; C- *M. phaseolina*; D- *Ph.columnaris*.

Results

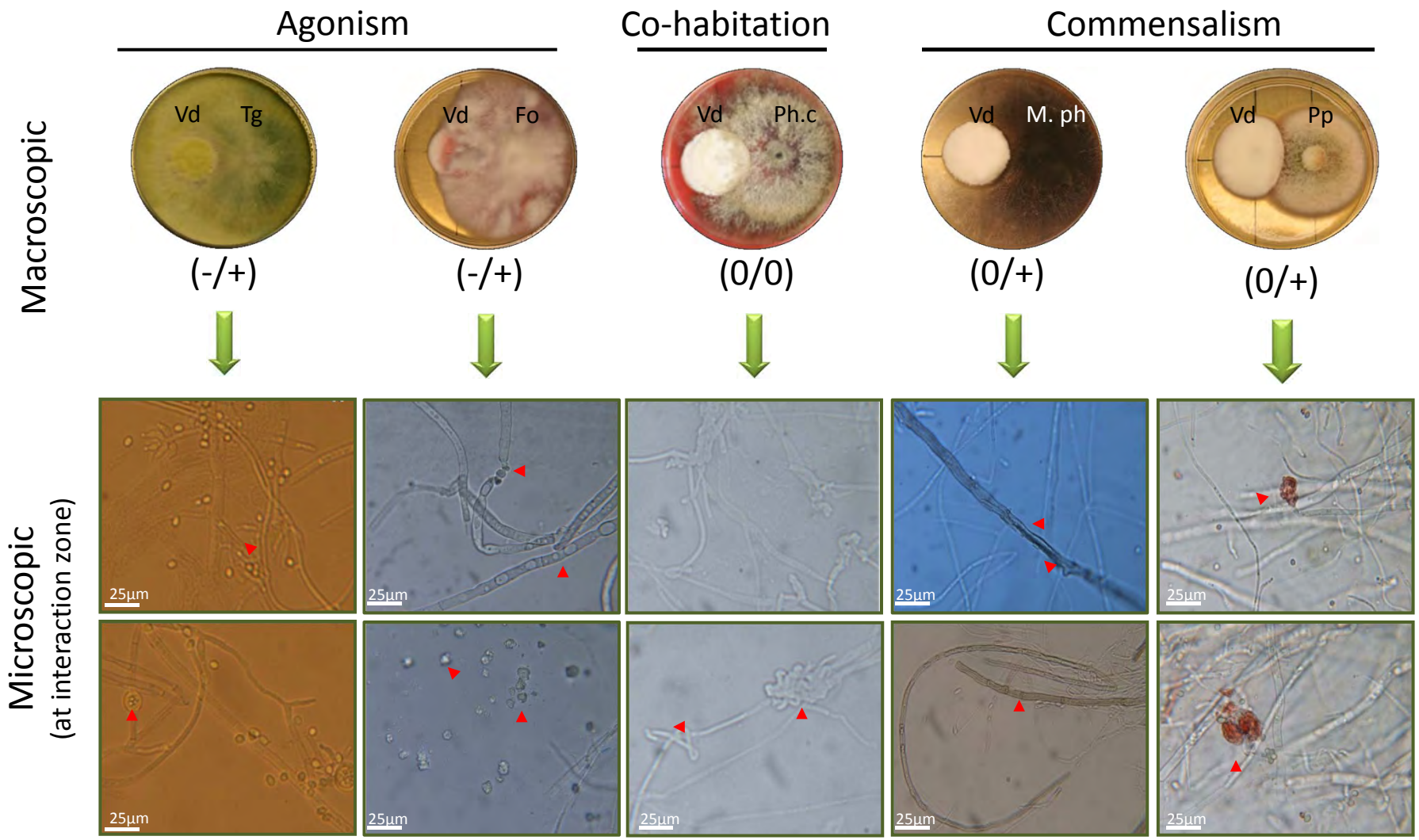
cv. Cobrançosa



Vd - Verticillium dahliae; Pr - Penicillium roseopurpureum; Pl - Paecilomyces lilacinus; Fo - Fusarium oxysporum; Hl - Hypocrea lixii; Pc - Penicillium commune.

Results

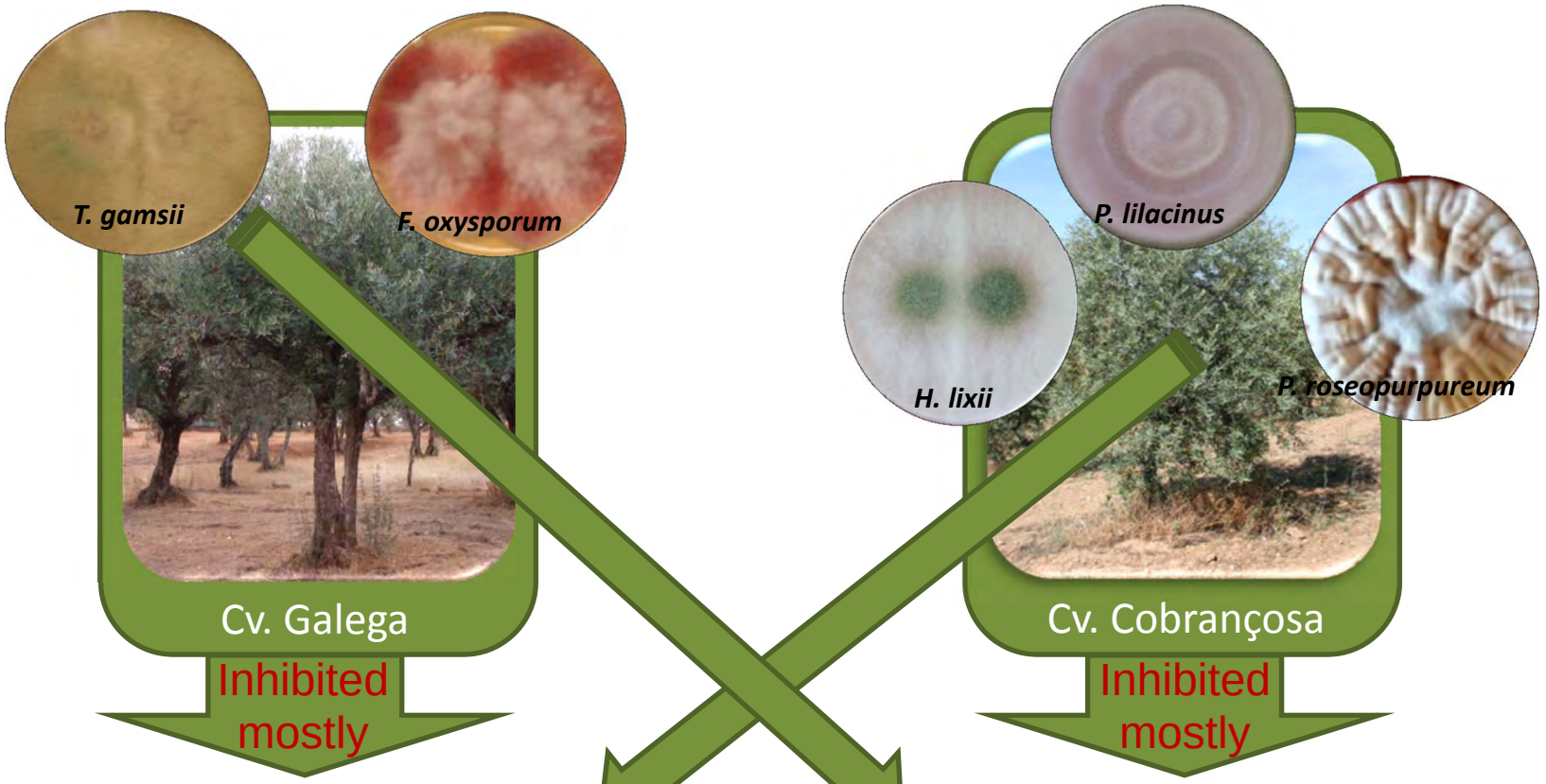
cv. Galega



Vd - Verticillium dahliae; Tg - Trichoderma gamsii; Fo - Fusarium oxysporum; Ph.c - Phomopsis columnaris; M. ph - Macrophomina phaseolina; Pp - Penicillium purpurogenum

Results

Fungal endophytes from:



Although preliminary, the results from the PCA analysis indicated that fungal endophytes could be responsible in part for disease resistance of cvs Cobrançosa and Galega to *V. dahliae* and *C. acutatum*

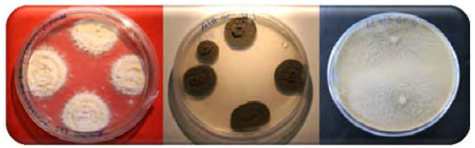
Verticillium dahliae

Colletotrichum acutatum

INSTITUTO POLITÉCNICO DE BRAGANÇA
Escola Superior Agrária

Project PTDC/AGR-AAM/102600/2008

Biological control of pests



i) To identify EF species associated to *Prays oleae*

ii) To evaluate the pathogenicity of EF autochthonous isolates

iii) To evaluate the effect of soil tillage in EF abundance and diversity

Ivo Oliveira
IPB/ESA

Procedure

Identification of EF species associated to *Prays oleae*

Sampling of *P. oleae* larvae and pupae

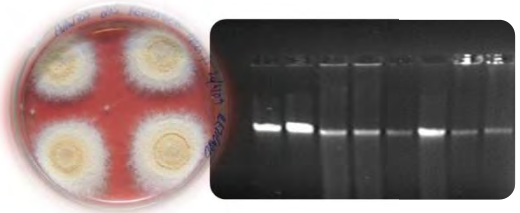
In three generations of this lepidopteran

Placed in sterile tubes

Daily checked for signs of fungal development

Isolation of fungi (PDA)

Amplification and sequencing of ITS region of the rDNA



Evaluation of the best DNA barcode region

Results

- 43 species 24 genera 14 families

Microb Ecol
DOI 10.1007/s00248-011-9955-z

HOST MICROBE INTERACTIONS

Fungal Diversity Associated to the Olive Moth, *Prays Oleae* Bernard: A Survey for Potential Entomopathogenic Fungi

Ivo Oliveira · José A. Pereira · Teresa Lino-Neto · Albino Bento · Paula Baptista

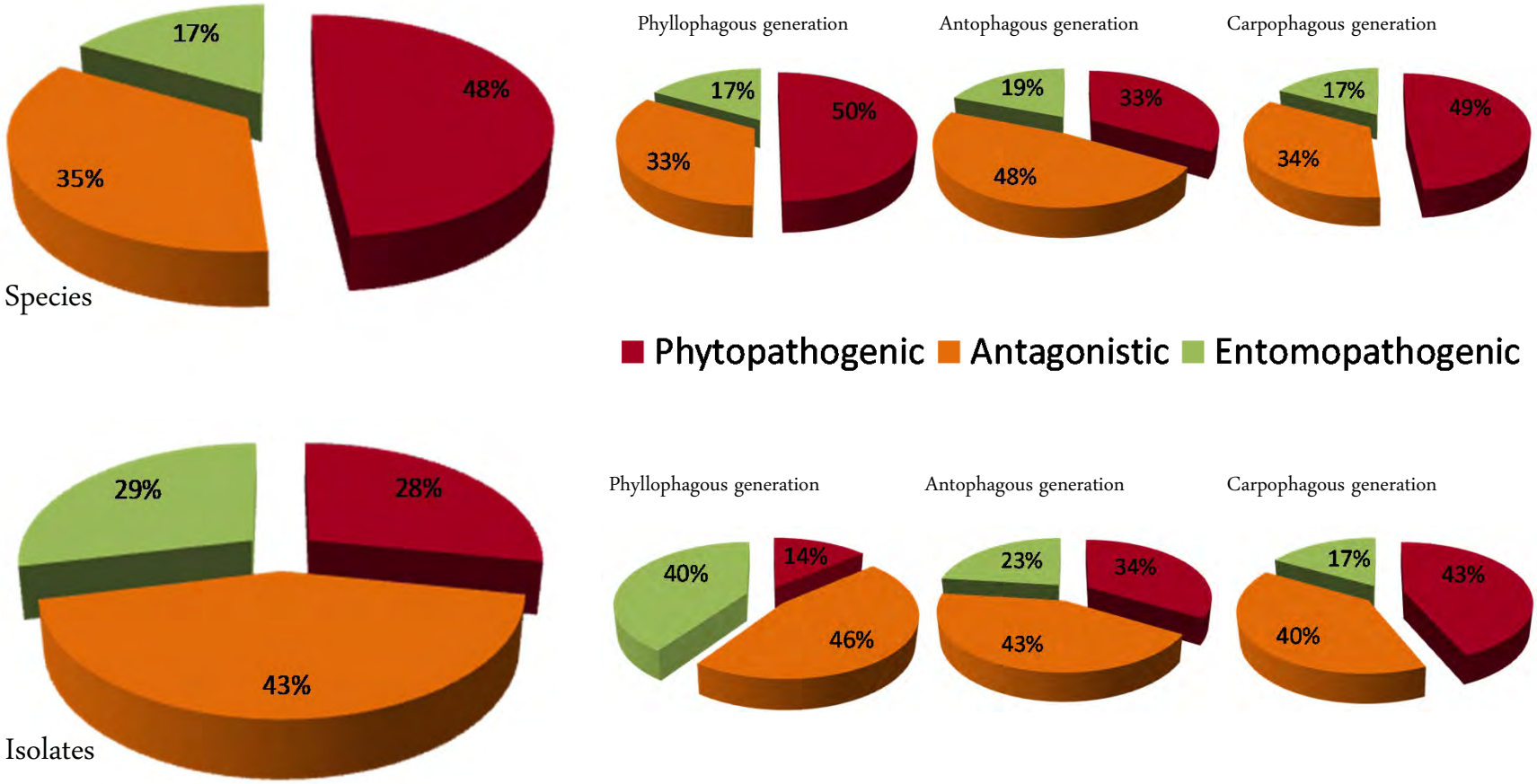
Olive moth generation	Number of species	More abundant (nº of isolates)
Phyllophagous	16	*B. bassiana,* N=49; 70.0%
Antophagous	13	*F. oxysporum,* N=23; 44.2%.
Carpophagous	21	*P. pinophilum,* N=7; 15.9%
Total	43	*B. bassiana,* N=50; 30.1%

Generation	Diversity indexes	
	D	*H*
Phyllophagous	0.50	1.39
Antophagous	0.25	1.68
Carpophagous	0.09	2.70

Higher fungal abundance and diversity in the carpophagous generation

Results

- Ecological roles



Variations of ecological roles proportion between *P. oleae* generation

Results

- Identified fungal species described as entomopathogenic

Species	Phyllophagous		Antophagous		Carpophagous	
	N	%	N	%	N	%
Aspergillus ustus (Bainier) Thom & Church	0	0.0	0	0.0	6	13.6
Beauveria bassiana (Bals.-Criv.) Vuill	49	70.0	0	0.0	1	2.3
Cladosporium cladosporioides (Fresen.) G.A. de Vries	1	1.4	0	0.0	1	2.3
Cordyceps sinensis (Berk.) Sacc.	1	1.4	0	0.0	0	0.0
Fusarium oxysporum Schltdl.	2	2.9	23	44.2	0	0.0
F. solani (Mart.) Sacc.	0	0.0	1	1.9	0	0.0
Fusarium sp.	0	0.0	1	1.9	0	0.0
Mucor circinelloides Tiegh.	0	0.0	0	0.0	3	6.8
M. hiemalis Wehmer	0	0.0	1	1.9	0	0.0
Penicillium sp. 1	0	0.0	0	0.0	1	2.3
Penicillium sp. 2	0	0.0	0	0.0	1	2.3

Species found exclusively in one of the generations; variation of the most abundant specie between generation

Procedure

Evaluation of the pathogenicity of EF autochthonous isolates

Preparation of conidial suspensions of 4 isolates of *B. bassiana*

A39GF09 **A67**GF09

LC39GF10 **PA95**GF10

1 mL of six concentrations (10^5 to 10^8)

Round plastic containers with sterile filter paper

Fourth instar larvae of *Cydia splendana*

Confirmation of *B. bassiana* induced mortality by re-isolation

Mortality of larvae, every 5 days, for 25 days

Median lethal time (LT_{50}) and median lethal concentration (LC_{50})

Results

- Virulence of *B. bassiana* isolates towards *C. splendana*

Isolates	LC_{50} (conidia/mL)	LT_{50} (10^5 conidia/mL)	LT_{50} (10^8 conidia/mL)
A39GF09	2.13 x 10^5 (8.23 x 10^4 - 4.13 x 10^5)	> 25	10.2 (9.2 - 11.2)
A67GF09	1.43 x 10^5 (5.31 x 10^4 - 2.81 x 10^5)	> 25	< 5
LC39GF10	< 1 x 10^5 (1.67 x 10^4 - 1.21 x 10^5)	12.6 (8.4 - 17.9)	< 5
PA95GF10	1.28 x 10^5 (5.25 x 10^4 - 2.36 x 10^5)	20.5 (12.4 - 28.3)	< 5

High virulence towards *C. splendana*, but with variations between isolates

Results

Evaluation of the effect of soil tillage in EF abundance and diversity

Sampling of larvae and pupae of *P. oleae* from orchards **with** or **without tillage**

Contents lists available at SciVerse ScienceDirect
Scientia Horticulturae
journal homepage: www.elsevier.com/locate/scihorti

Effect of soil tillage on natural occurrence of fungal entomopathogens associated to *Prays oleae* Bern.

Ivo Oliveira[a], José A. Pereira[a], Enrique Quesada-Moraga[b], Teresa Lino-Neto[c], Albino Bento[a], Paula Baptista[a,*]

- Diversity indexes

		D	*H*
Generation	Phyllophagous	0.54 ± 0.18 a	0.80 ± 0.30 a
	Antophagous	0.90 ± 0.22 b	0.14 ± 0.31 b
	Carpophagous	0.75 ± 0.29 b	0.35 ± 0.40 b
Soil type	Tillage	0.40 ± 0.04	1.09 ± 0.13
	No tillage	0.36 ± 0.15	1.26 ± 0.35
Phyllophagous	Tillage	0.54 ± 0.24	0.83 ± 0.39
	No tillage	0.53 ± 0.16	0.78 ± 0.27
Antophagous	Tillage	1.00 ± 0.00	0.00 ± 0.00
	No tillage	0.75 ± 0.25	0.35 ± 0.35
Carpophagous	Tillage	0.50 ± 0.00	0.69 ± 0.01
	No tillage	0.83 ± 0.29	0.23 ± 0.40

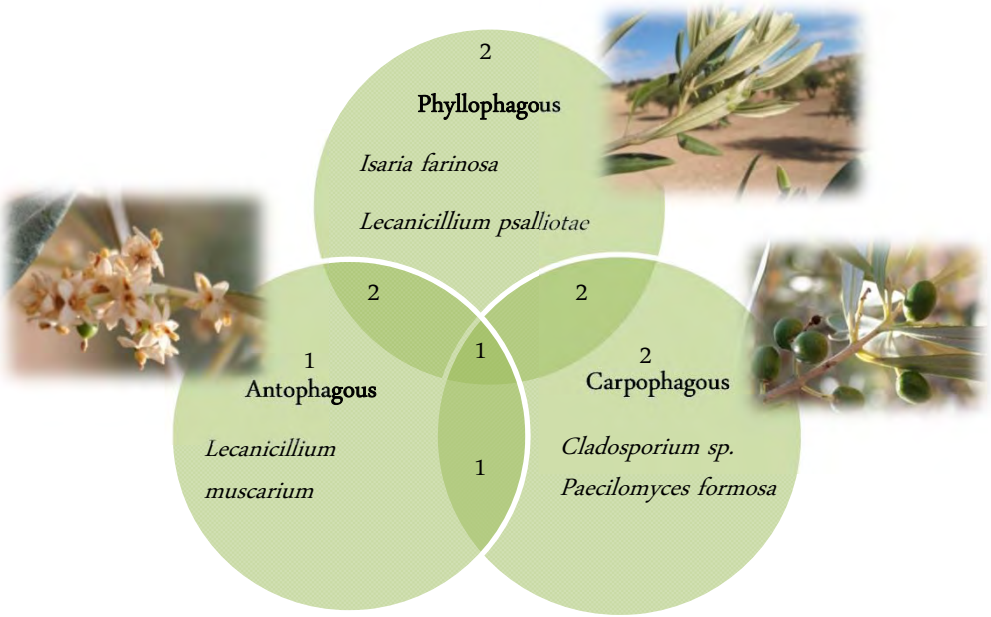
Significant differences

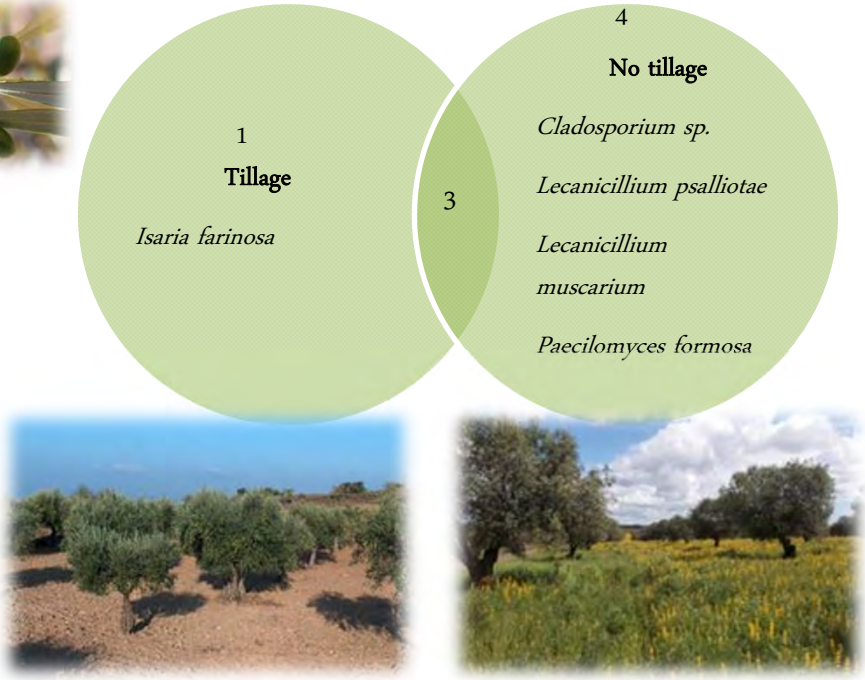
No significant differences

No significant effects of soil tillage on fungal diversity

Results

- Species exclusive to one generation/soil type





Future prospects

Continuous monitoring of the influence of biotic and abiotic factors

Selection of the most suitable isolates

Mass production trials

Field trials, testing both formulations and application methods

Production of mycoinsecticides

Acknowledgments

COMPETE

QUADRO DE REFERÊNCIA ESTRATÉGICO NACIONAL

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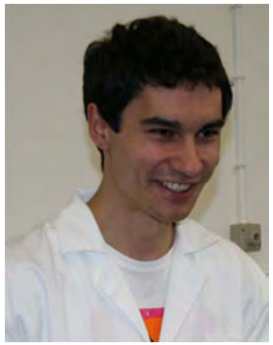

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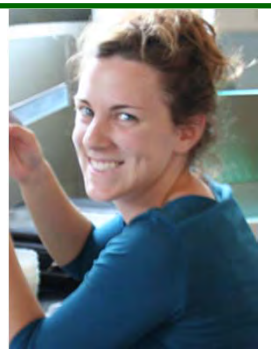

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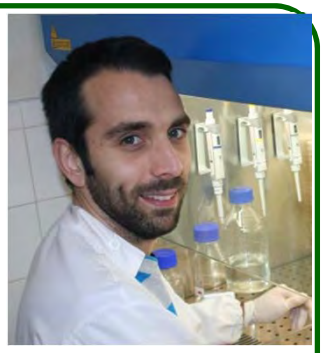

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