CITAB

Centro de Investigação e de Tecnologias Agro-ambientais e Biológicas

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1º Simpósio SCAP

e 7º Congresso da SPF

On the occurrence of entomopathogenic fungi and probable gut inhabiting yeast in vine mealybug *Planococcus ficus* (Signoret) (Hemiptera: Pseudococcidae).

Sobre a ocorrência de fungos entomopatogénicos e de leveduras hipoteticamente residentes no intestino da cochonilha-algodão-da-vinha, *Planococcus ficus* (Signoret) (Hemiptera: Pseudoccidae)

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Projecto EcoVitis: Maximização dos serviços do ecossistema vinha na Região Demarcada do Douro

MEALYBUGS

- The mealybug family (Hemiptera: Pseudococcidae).
- 2020 species from 300 genera (Ben-Dov & Miller, 2012)
- Name ‘mealybug’ from white mealy wax.
- Mealybugs are all phytophagous (Vieux *et al*., 2013).

All Images from Daane, *et al*., 2008 Vineyard managers and researchers seek sustainable solutions for mealybugs, a changing pest complex. California Agriculture, 62: 167-176.

Ben-Dov, Y. & Miller, D.R., 2012. ScaleNet: A database of scale insects of the world. ; D. **le Vieux** & A.P. Malan, 2013, *S. Afr. J. Enol. Vitic*., 34(1), 108-118

MEALYBUGS IN VINEYARDS

SPECIES AND AUTHOR	ORIGIN	CURRENT PRESENCE
Pseudococcus Maritimus (Ehrhorn)	Nearctic	North America
Pseudococcus viburni (Signoret)	Neotropic	Australia, Europe, New Zealand, N. America (California), South Africa , S. America
Pseudococcus longispinus (Targioni-Tozzetti)	Australasia	Australia, Europe, New Zealand , California, S. Africa, S. America
Pseudococcus calceolariae (Maskell)	Australasia	Australia, Europe, New Zealand , N. America, S. Africa, S. America
Planococcus citri (Risso)	Palearctic	Australia, Europe, New Zealand , N. America, S. Africa, S. America
Planococcus ficus (Signoret)	Palearctic	-discussed later-
Dysmicoccus brevipes (Cockerell)	Indo- Malaya	Australia, Africa, Asia, Middle East, S. America (Brazil)
Ferrisia gilli (Gullan)	Nearctic	California
Maconellicoccus hirsutus (Green)	Indo- Malaya	Australia, Africa, Asia (India), Middle East, S. America, Mexico, California

Adapted from: Daane, K. M., R.P.P. Almeida, V. A. Bell, M. Botton, M. Fallahzadeh, M. Mani, J. L. Miano, R. Sforza, V. M. Walton, and T. Zaveizo. 2012. Biology and management of mealybugs in vineyards, pp. 271–308. *In*N. J. Bostanian, R. Isaacs, and C. Vincent (eds.) Arthropod Management in Vineyards. Springer, The Netherlands.

VINE MEALYBUG: *Planococcus ficus*

- Very small (> 5 mm), soft-bodied insects.

- Piercing, sap-sucking mouthparts.

- Produce honeydew encouraging the growth of sooty mold on grapes.


Photo credits: Cristina Carlos

- Moreover, feeds on subtropical and tropical crops and common weeds.

- Transmitting plant viruses,

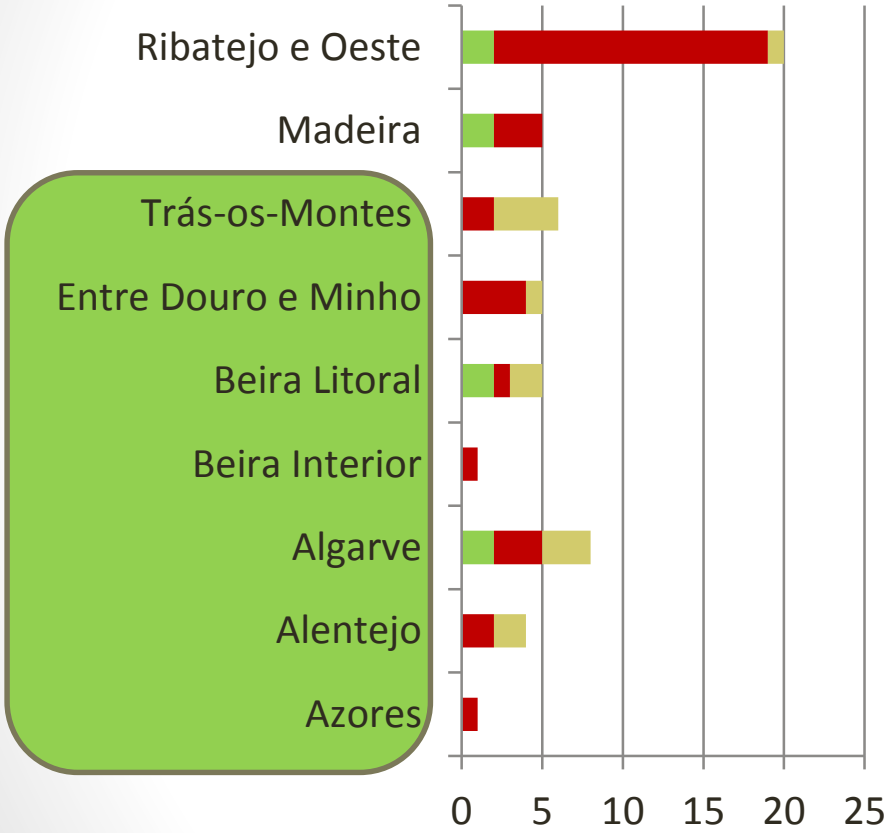
a) Grapevine virus A (GVA),
b) Grapevine virus B (GVB),
c) Grapevine leafroll-associated viruses (GLRaV) (Iasur-Kruh *et al*., 2014)


Photo credits: Ana Pereira

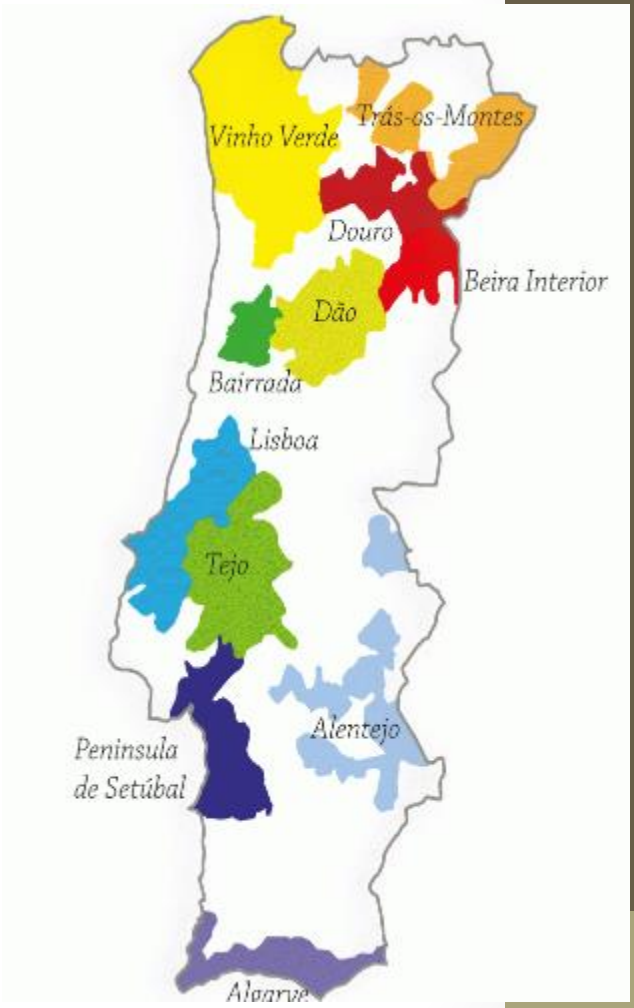
- Mediterranean regions of Europe, Africa, Middle East and Argentina California and Mexico

Iasur-Kruh *et al.,* 2014, *Microbial Ecology*;

MEALYBUGS IN PORTUGUESE VINEYARDS



P. ficus dominated regions



Map Source: Pinterest.com

Adapted from **Godinho** M.A. & **Franco** J.C. 2001. Survey on the pest status of mealybugs in Portuguese vineyards. IOBC/WPRS Bull. 24 (7): 221–225

CONTROLLING *P. ficus*

- **Chemical control:**

a) short-residual organophosphates (e.g. mevinphos); or

b) delayed dormant organophosphates (e.g. chlorpyrifos) (Daane *et al*., 2006)

Problems:

a) Hiding inside crevices where chemicals do not reach for e.g., under the bark and on the roots (Walton & Pringle, 2004).

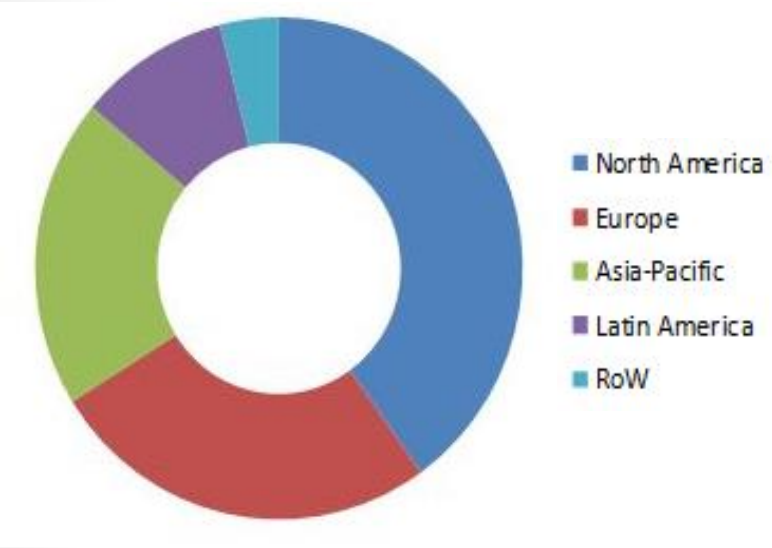
b) Unwanted killing of natural parasitoids (Mgocheki & Addison 2009).

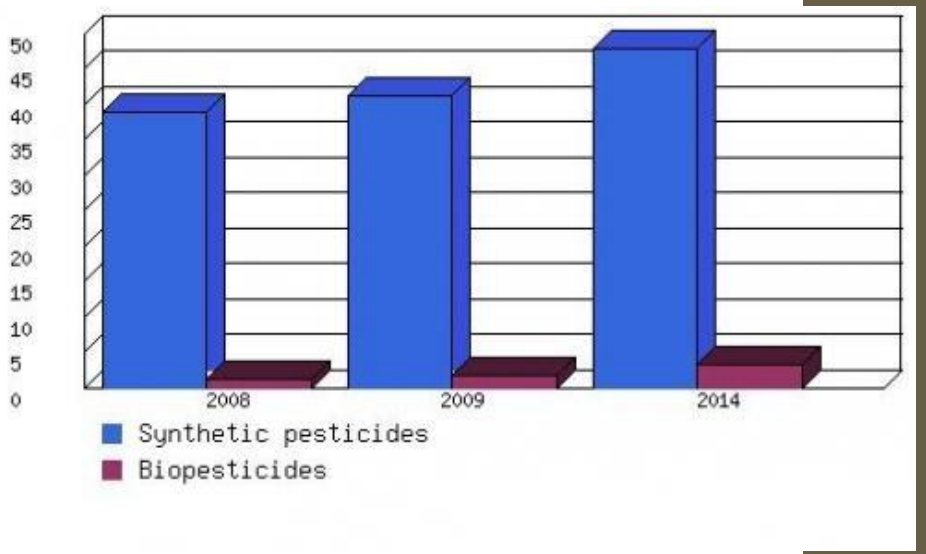
c) Hydrophobic waxy secretions interferes with insecticide penetration (Franco *et al*., 2009)

Daane, K.M., *et al.,* 2006. *Calif. Agr.* 60, 31-38. ; **Franco**, J.C., Zada, A. & Mendel, Z., 2009. Novel approaches for the management of mealybug pests. In: Ishaaya, I. & Horowitz, A.R. (eds). Biorational control of arthropod pests: Application and resistance management. Springer Science, New York. pp. 233 – 278. ; **Mgocheki** N., Addison P., 2009-. *S. Afr. J. Enol. Vitic*. 30, 110-116 ; **Walton**, V.M. & Pringle, K.L., 2004 *S. Afr. J. Enol. Vitic*. 25, 54-62.

ENTOMOPATHOGENS

Organisms killing insect pests. For e.g., bacteria, fungi, nematodes and virus.





Biopesticides Market Share, by Geography, 2013

Growth at 16.0%

$4,369.88 million by 2019.

$1.6 billion in 2009, projected increase: $3.3 billion in 2014

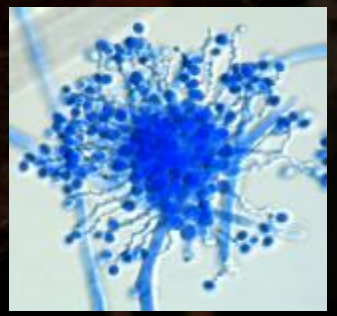
Source: Expert Interviews and Markets and Markets Analysis; BBC Research

ENTOMOPATHOGENIC FUNGI (EPF)

Over 700 species from 90 different genera;
around 170 products from 12 different species are commercialised

Species for e.g.,

Beauveria sp.
(*B. bassiana* ;
B. pseudobassiana,
B. brongniartiii);



Bionectria ochroleuca

Fusarium spp.

Metarhizium sp.
(*M. anisopliae*, *M. acridum*,
M. robertsii);

Beauveria bassiana: Infect 750 insect species approximately.

Vega *et al*., 2009 *Fungal Ecology,* 2 (4): 149-159.

500 mm

AIM OF THE PROJECT

TO UNDERSTAND DIVERSITY OF FUNGI ASSOCIATED WITH CADAVERS OF VINE MEALYBUG *PLANOCOCCUS FICUS* (SIGNORET) (HEMIPTERA: PSEUDOCOCCIDAE) COLLECTED FROM DIFFERENT FARMS OF DOURO WINE REGION OF PORTUGAL.

FARMS UNDER INVESTIGATION

Aciprestes

Arnozelo

Farms in study

Carvalhas

a) Raposeira **b) Placas**

Granja

Cidro

S. Luis

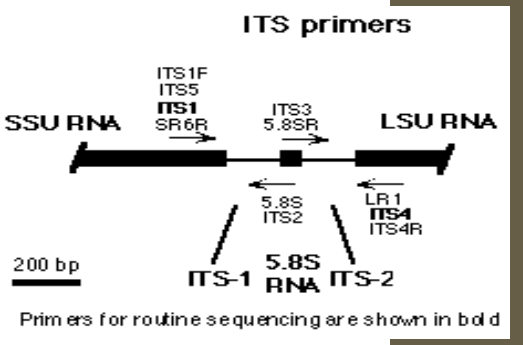
Vallado

PROCURING FUNGI FROM CADAVERS

- Spotting mycoses on mealybugs.
- Surface sterlisation by 1% Na-Hypochlorite for 3 minutes.
- Subsequent washings with sterlised water.
- Culturing onto selective media Dichloran Rose bengal Chloramphenicol Agar (Sigma-Aldrich Chemie, Buchs, Switzerland).
- Maintaining pure culture on PDA (Sigma-Aldrich Chemie, Buchs, Switzerland)

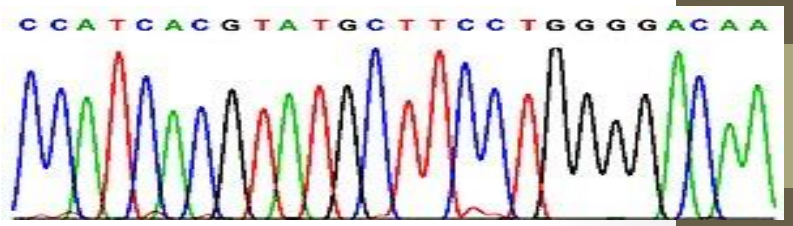
MOLECULAR IDENTIFICATION

- PDA cultures with cellophane sheets.



- DNA extraction using CTAB-lysis buffer.

- PCR amplification of ITS region between 18S and 28S rDNA using ITS1+ ITS4 primers (White *et al*., 1990)

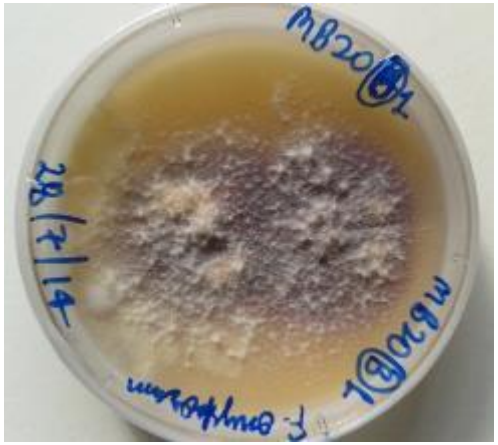


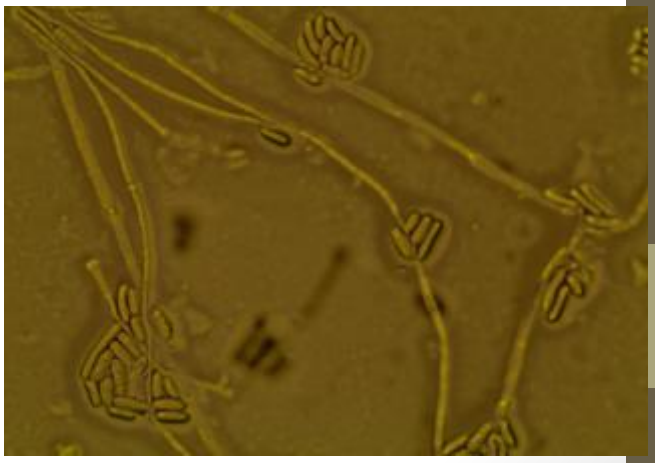
- BLAST the obtained sequence (http://blast.ncbi.nlm.nih.gov/Blast.cgi)

White *et al.,* 1990. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M. A., D. H. Gelfand, J. J. Sninsky, and T. J. White. Academic Press, Inc., New York.©W°

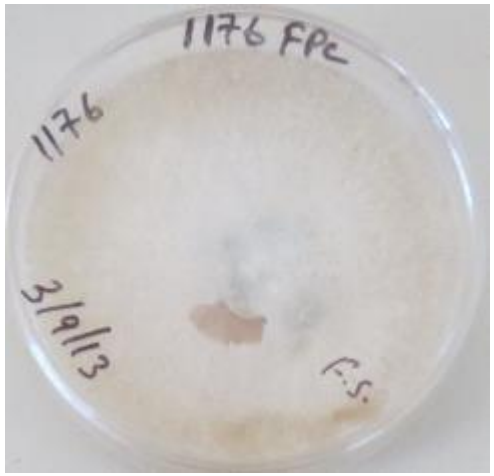
RESULTS: MICROSCOPIC IDENTIFICATION

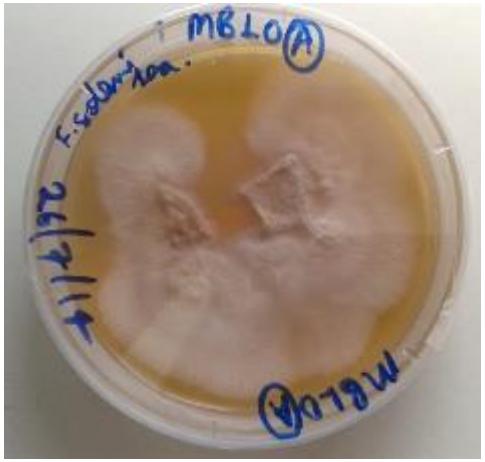
3 isolates of *Fusarium oxysporum* species complex



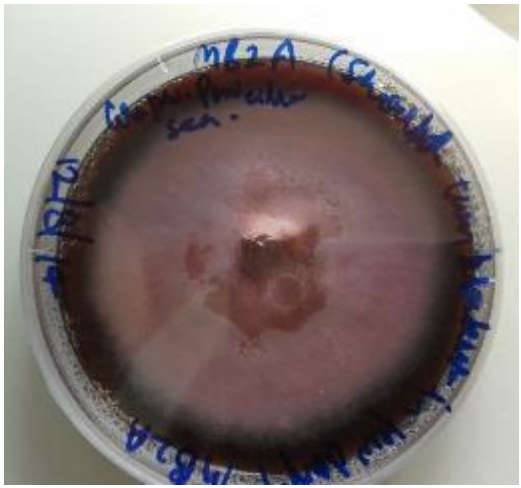


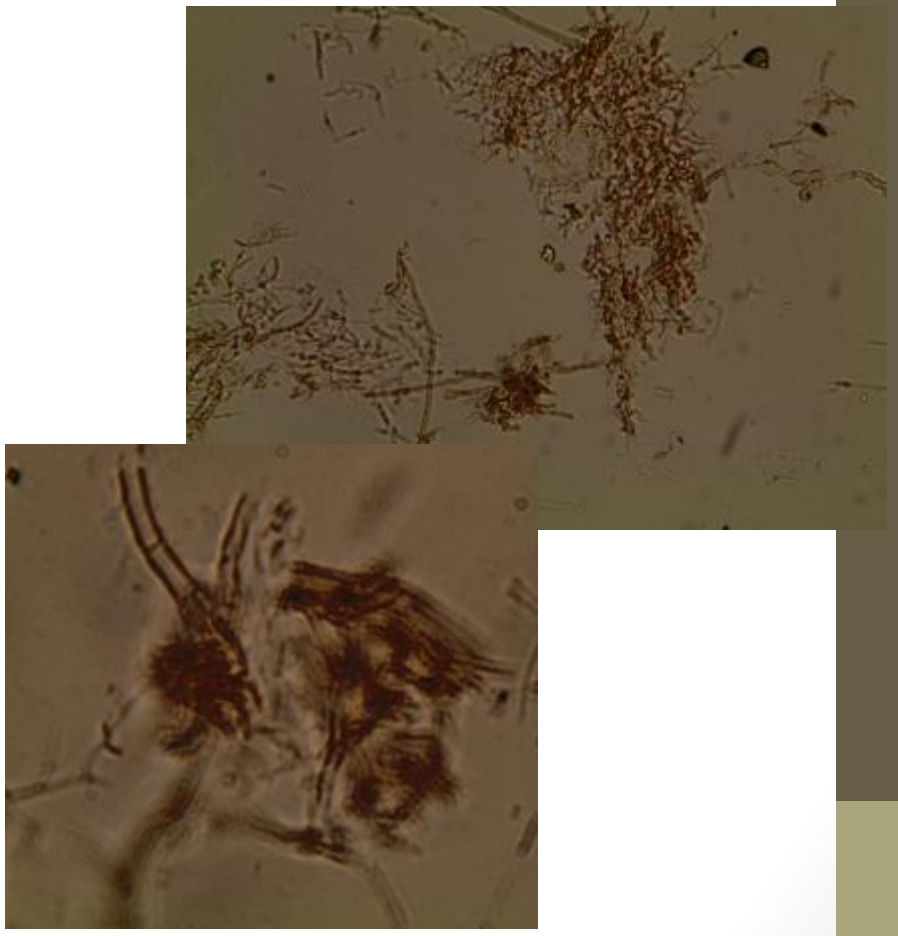
1 isolate of *Fusarium solani* species complex



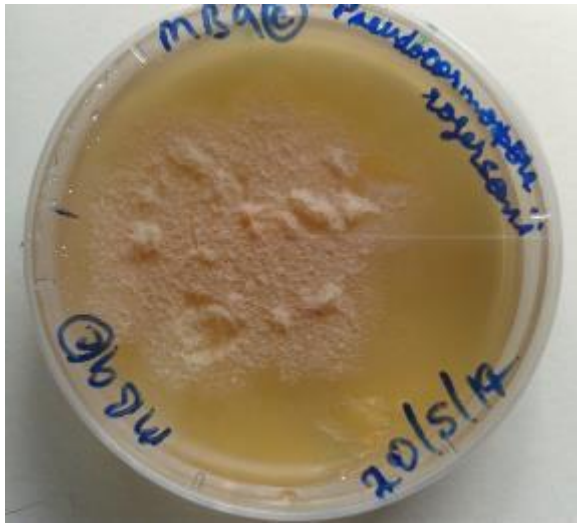


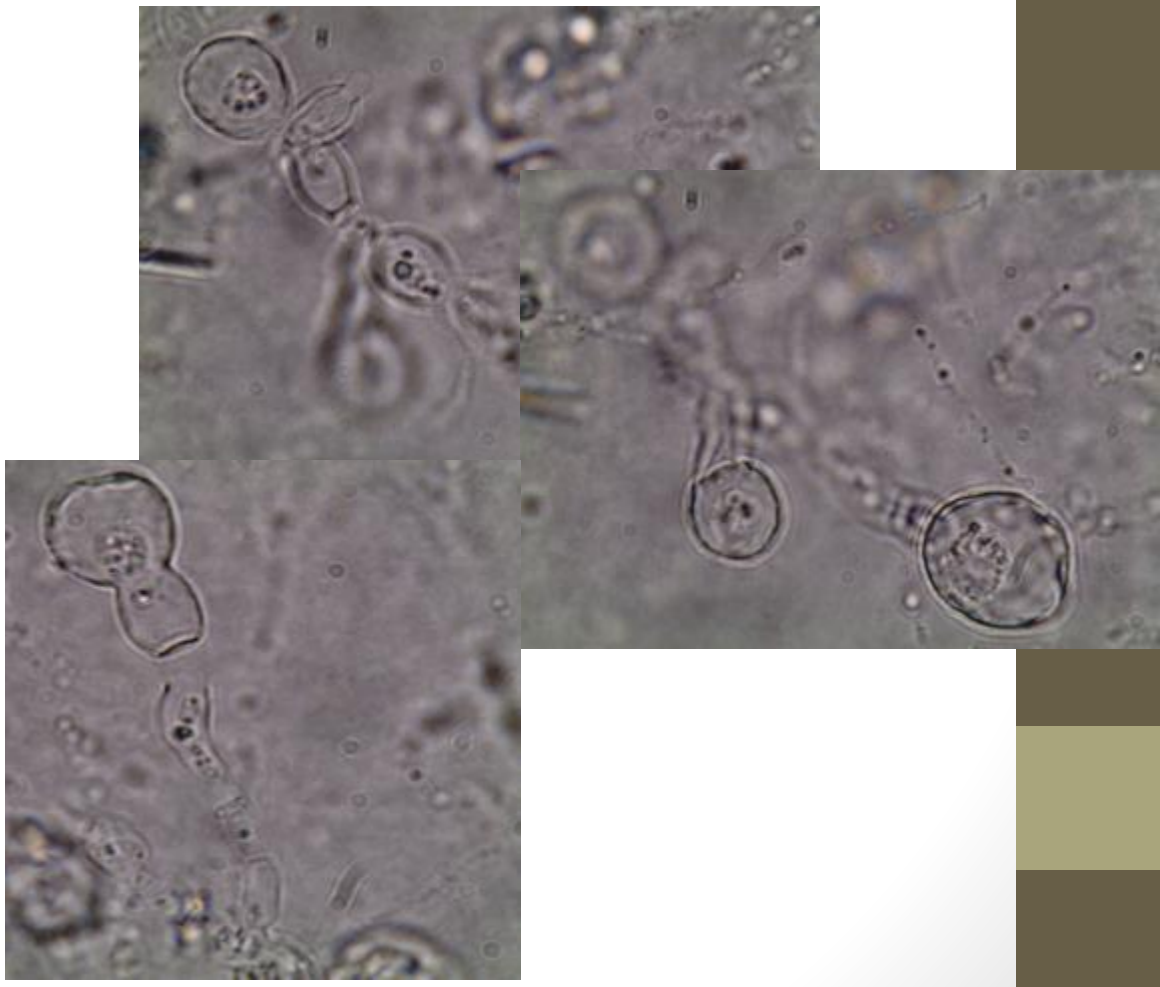
1 isolate of *Graphium penicillioides*





1 isolate of *Pseudocosmospora rogersonii*

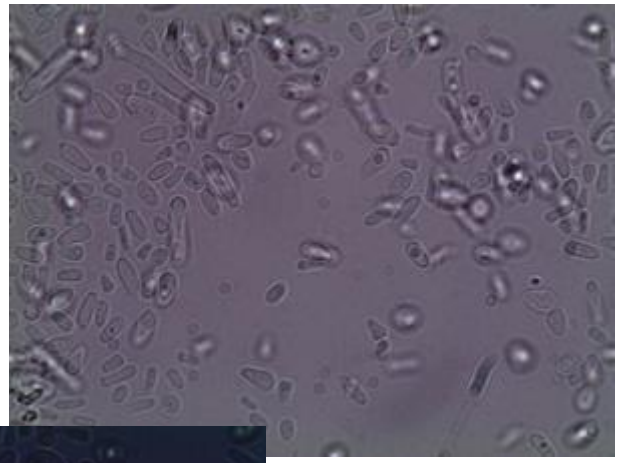


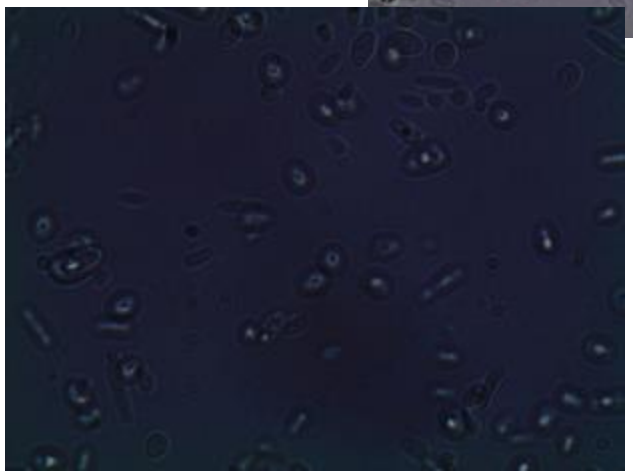


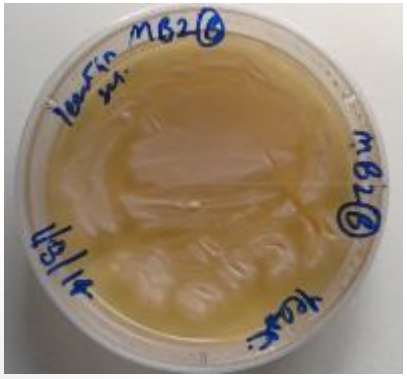
1 isolate of *Penicillium chrysogenum*

4 isolates of yeast

Pichia (= *Meyerozyma*) (anamorph *Candida*) *guilliermondii*



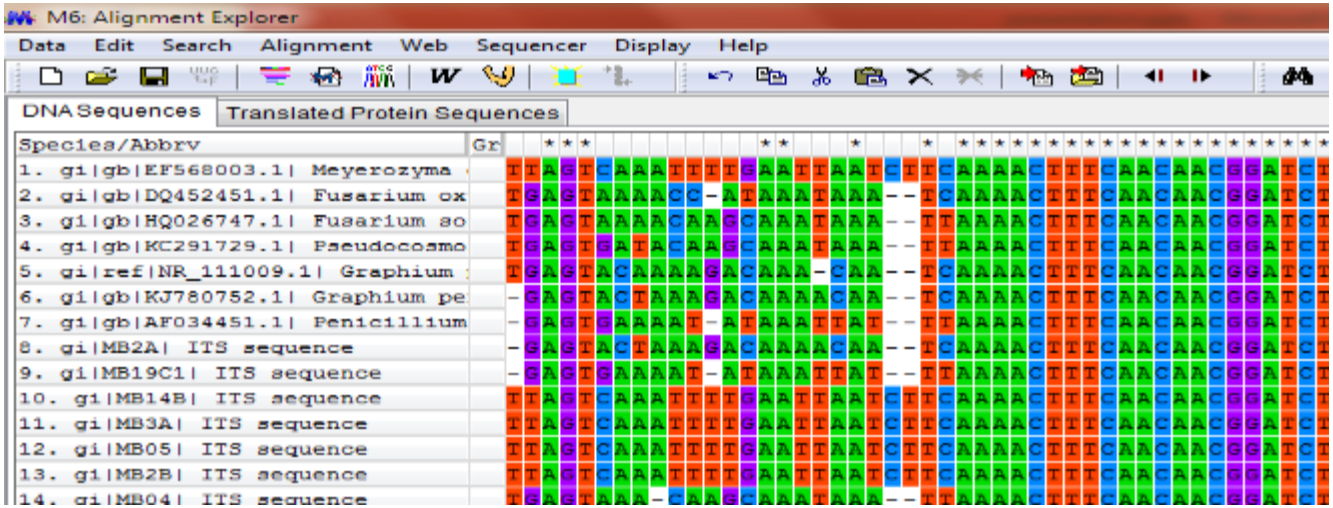




MOLECULAR DATA ANALYSES

- Editing gene sequences for corrections in Bioedit, version 7.1.3.0 (Hall, 1999).

- Sequence alignment using Clustal W in Mega 6.0 (Larkin *et al*., 2007 Tamura *et al*., 2013)



Hall, T.A. 1999. *Nucl. Acids. Symp. Ser*. 41:95-98. ; **Larkin** *et al*., 2007 *Bioinformatics 23(21): 2947-2948. ;* **Tamura** K. *et al*., 2013. *Molecular Biology and Evolution* 30: 2725-2729.

GLIMPSES INTO PHYLOGENETIC PLACEMENT

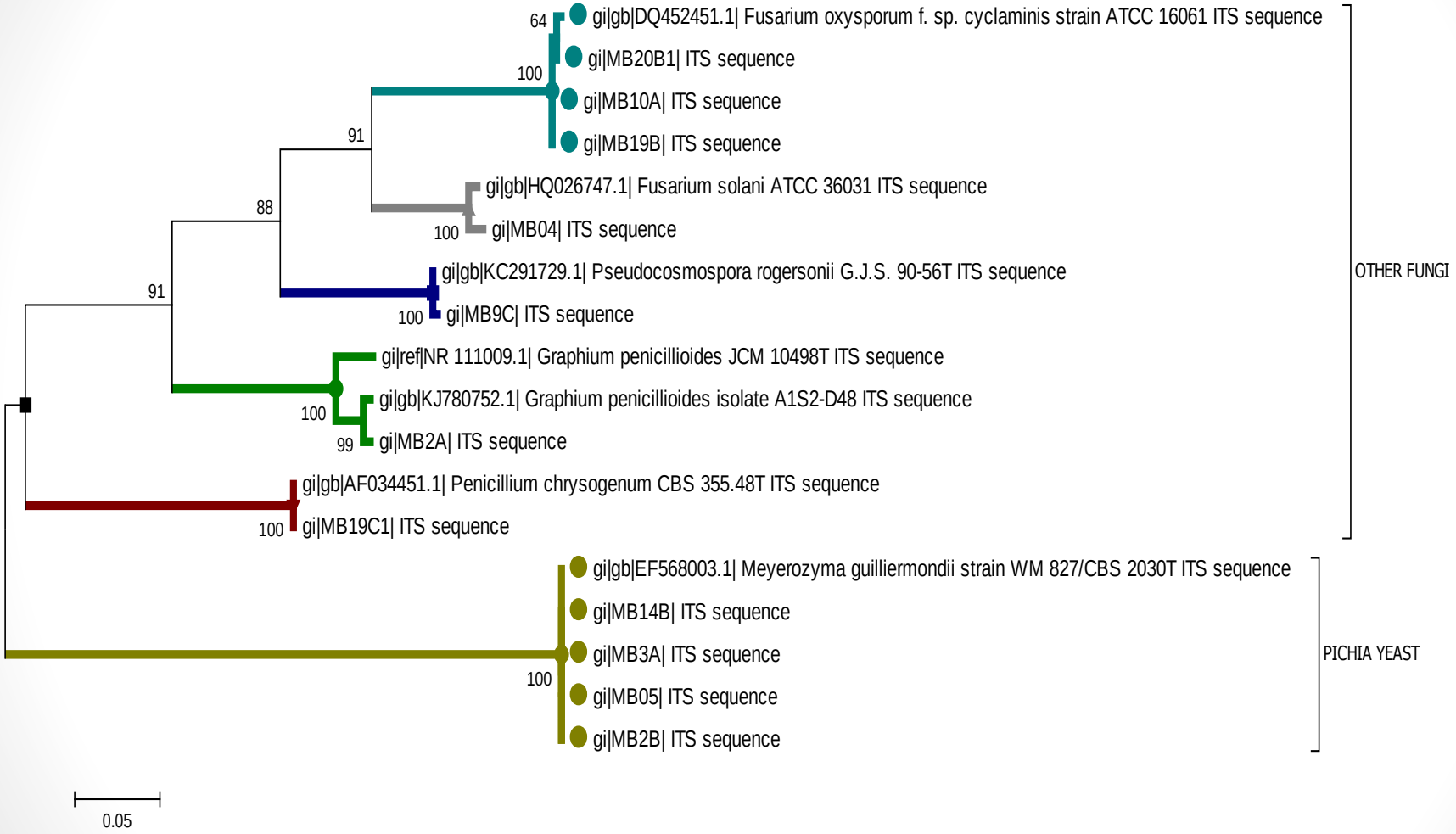


Figure. Molecular Phylogenetic analysis by Maximum Likelihood method The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [1]. The tree with the highest log likelihood (-2000.5694) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 18 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 380 positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

1. Tamura K. and Nei M. (1993). Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology and Evolution 10:512-526.

DISCUSSION

Fungal infection on mealybugs:

EPF	MEALYBUG	REFERENCE
Field reports on mycoses		
Cephalosporium sp.	*Planococcus citri* (Citrus mealybug)	Rogter *et al*., 1960
Hirsutella cryptosclerotium sp. nov.	*Rastrococcus invadens*	Fernández-García *et al*., 1990
Neozygites fumosa	*Phenacoccus herreni* (Cassava Mealybug)	Delalibera *et al*., 1990
Laboratory reports on bio-control		
Hypocrella hypocreoidea; Metarhizium anisopliae & Fusarium solani	*Pseudococcus cryptus*	Panyasiri *et al*., 2007
Isaria farinosa	*Planococcus citri,* citrus mealybug	Demirci *et al*., 2011
Lecanicillium lecanii	*Phenacoccus solenopsis*	Kumar *et al*., 2012

Rogter *et al*., 1960, *Ghana Journal of Science,* 6, 110-114; **Delalibera** *et al.,* 1990, *J. Invertebr. Pathol ,* 276–278.69.1997.; **Fernández-García** *et al*. 1990,. *Mycological Research* 94(8), 1111–1117; **Kumar** *et al*., 2012,, *Journal of Entomology,* 9(1), 32-40 ; **Demirci** *et al*., 2011**,** *Journal of Pest Science*, 84(3), 337-342. **Panyasiri** *et al*., 2007, *Journal of Plant Diseases and Protection*, **114** (6), 278–287

OBTAINED FUNGI ??

- Many studies reports mortality of Insects from

a) *Fusarium oxysporum* &

b) *F. solani* (Ali-Shtayeh *et al*., 2003; Oliveira *et al*., 2012)

- *Graphium* sp. also found from bark beetles (Wingfield & Gibbs, 1991; Mouton *et al*., 1994)

Wingfield, M.J. & Gibbs, J.N. 1991, *Mycological Research,* 95(11), 1257–1260. ; **Mouton** *et al*., 1994 *Mycological Research* 98(11), 1272–1276
Ali-Shtayeh, M.S., *et al*., 2003 *Mycopathologia* 156, 235– 244; **Oliveira**, I. *et al*., 2012. *Microbial Ecology* 63, 964-974

CONSIDERING LESSER EPF AVAILABILITY

- Presence of yeasts in different life stages of *P. ficus*

a) *Pichia* (= *Meyerozyma*) (anamorph *Candida*) *guilliermondii*

b) *Metschnikowia pulcherrima*

c) *Rhodotorula mucilaginosa*

(in this study & Iasur-Kruh *et al*., 2014)

with antifungal actions (Sharma *et al*., 2009; Zhang *et al*., 2013).

Other possible factor: Acidic microenvironment (Ashbolt & Inkerman, 1990).

Sharma *et al.,* 2009, *Biological Control,* 50, 205–221; **Iasur-Kruh** *et al.,* 2014, *Microbial Ecology*; **Zhang** *et al.,* 2013, *Carbohydrate Polymers* , 98, 366–371
Ashbolt N.J. & Inkerman P.A. ,1990, *Appl. Environ. Microbiol.* 56(3):707-712;

RESEARCH HIGHLIGHTS

According to our knowledge,

a) first study on isolation of cadaver associated fungi from *P. ficus.*

b) first report on isolation of *Pseudocosmospora rogersonii* from Southern Europe.

c) our hypothesis of *M. guilliermondii* residing in *P. ficus* matched with very recent findings of Lilach Iasur-Kruh and coworkers (Microbial Ecology, 2014)

d) isolation of another yeast *Rhodotorula mucilaginosa* from eggs of *P. ficus* feeding on grapevine.

CONCLUSION AND FUTURE WORKS

- Insights into possible microbial biocontrol agents for *P. ficus.*

- FUTURE WORKS:

a) To investigate possible roles of isolated yeasts in fungal infection on *P. ficus*.

b) To screen the most effective fungus for biocontrol of *P. ficus.*

- OBRIGADO PELA ATENÇÃO

Co-financiamento: Programa de Desenvolvimento Rural – Ministério da Agricultura, Mar, Ambiente e Ordenamento do Território – Fundo Europeu Agrícola de Desenvolvimento Rural – A Europa investe nas zonas rurais