Role of *B. bassiana* on Plant Defence, Biocontrol and Insect Behaviour modification

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High-Level Meeting on Red Palm Weevil Management
Rome, Italy, 29-31 March, 2017

Palms form oases of high environmental and economical value and are Important cultural/touristic assets

Alicante

PATRONATO MUNICIPAL DE TURISMO Y PLAYAS

Elche

Oasis Mediterráneo

RPW is major palm pest worldwide...

RPW can damage Date Palm Value

Biocontrol: Entomopathogenic fungi

B. bassiana: most common Entomopathogenic Fungus in dry Ecosystems (SE Spain)

Aphids

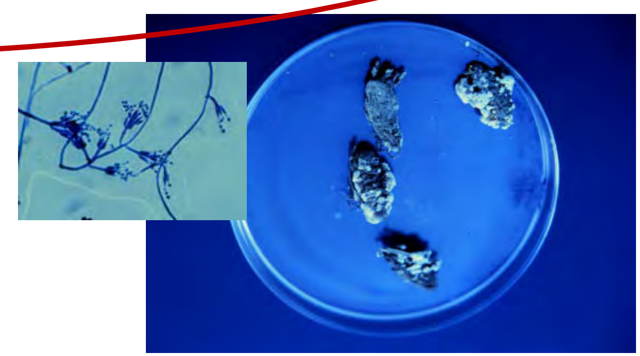
Entomophthorales
(*Erynia neoaphidis*)

Mitosporic fungi

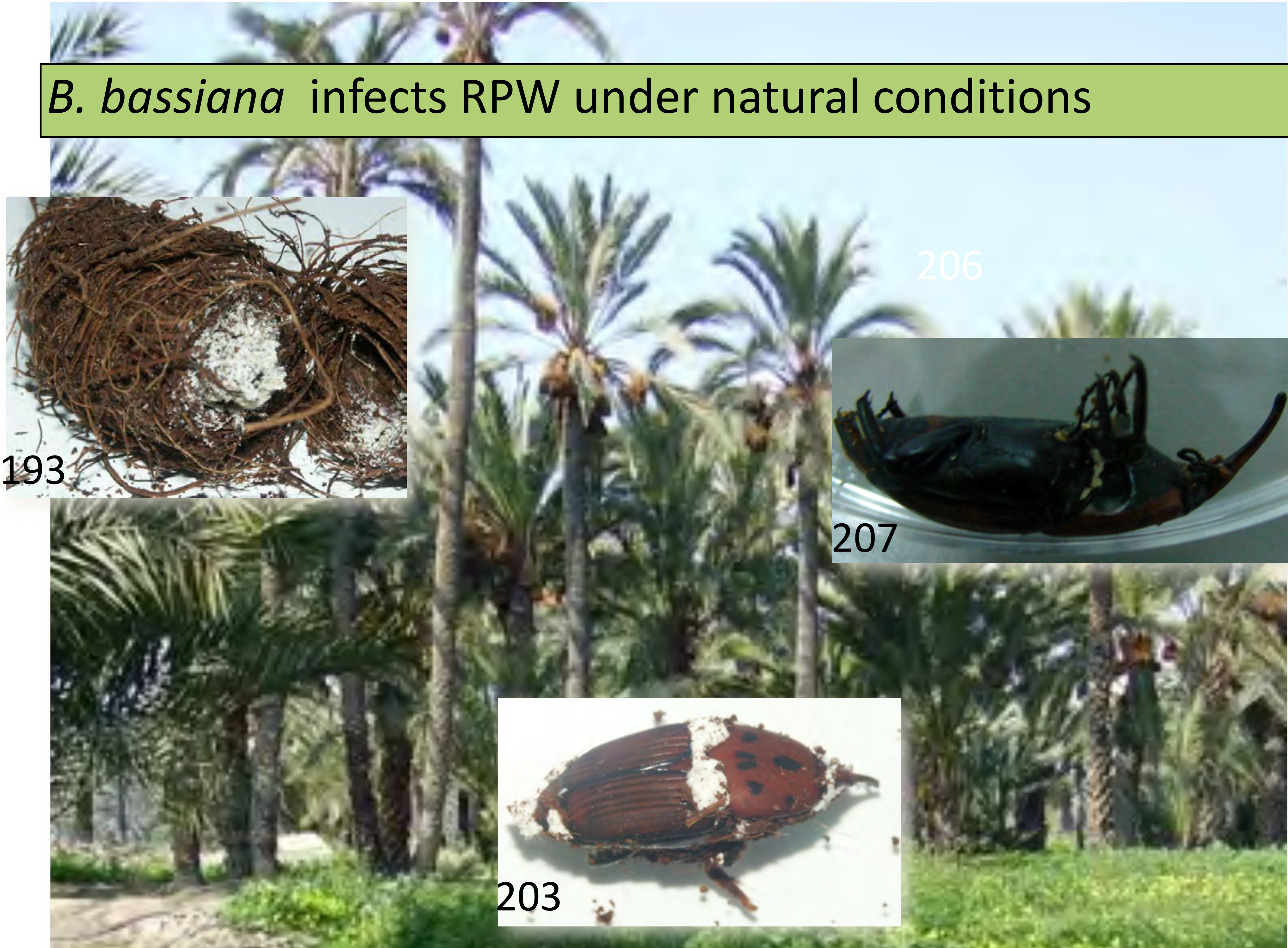
Coleopterans (*Beauveria bassiana*)

Thrips

Lepidopterans
(*Paecilomyces farinosus*)



B. bassiana infects RPW under natural conditions



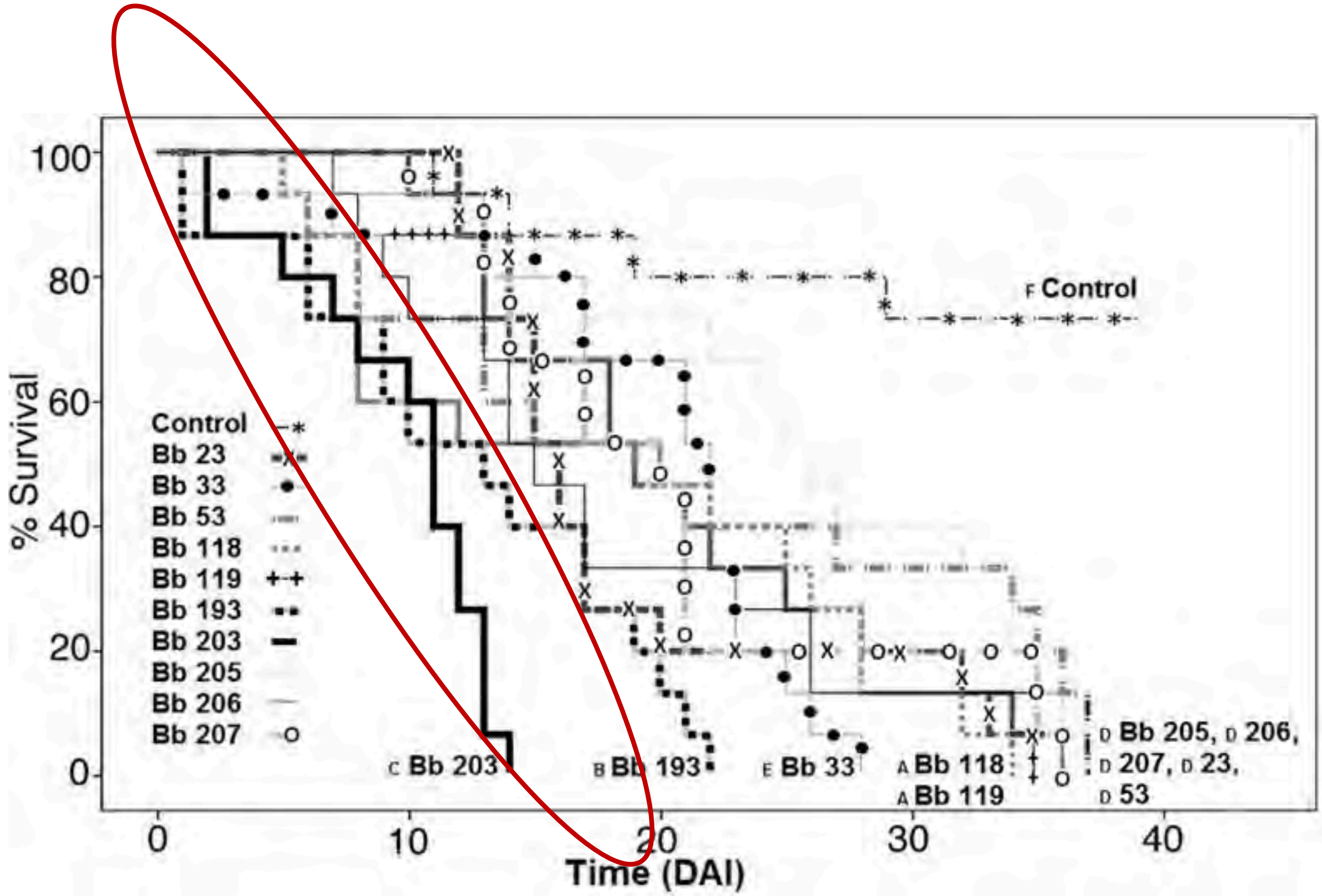
193

206

207

203

Bb 203 is the most pathogenic strain to RPW



Ricaño, J., Güerri-Agulló, B., Serna-Sarriás, M. J., Rubio-Llorca, G., Asensio, L., Barranco, P., & Lopez-Llorca, L. V. (2013). Evaluation of the pathogenicity of multiple isolates of Beauveria bassiana (Hypocreales: Clavicipitaceae) on Rhynchophorus ferrugineus (Coleoptera: Dryophthoridae) for the assessment of a solid formulation under simulated field conditions. *florida entomologist*, *96*(4), 1311-1324.

Universitat d'Alacant
Universidad de Alicante

Bb 203 can be dry formulated and applied to target RPW in the field

B. bassiana dry conidia are captured by RPW cuticle

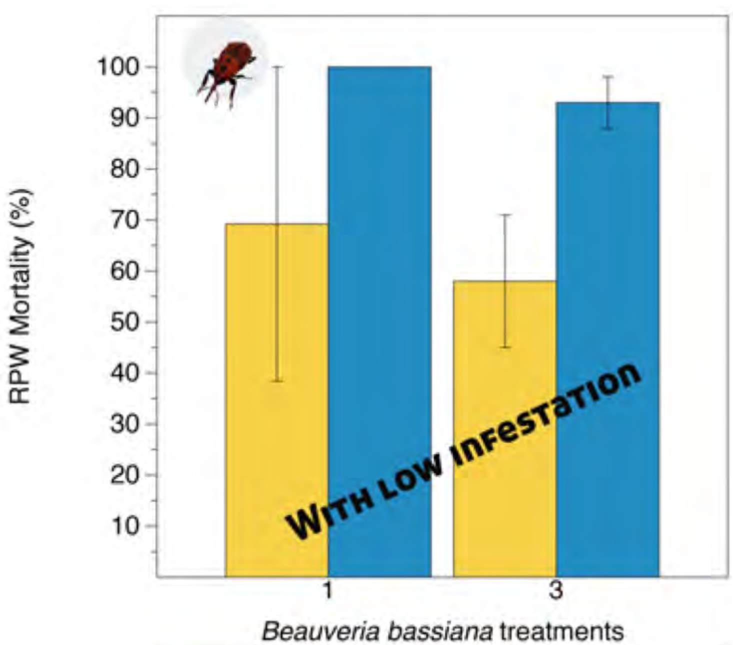

B. bassiana dry conidia germinate, adhere…
SE
WD10.7mm 20.0kV x3.5k 10um

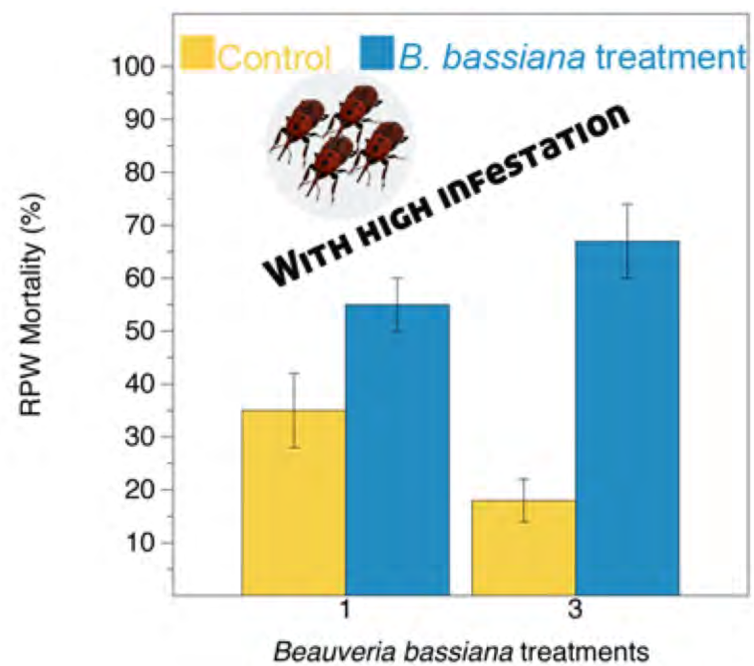
… and penetrate RPW cuticle

Güerri-Agulló et al. (2010)

B. bassiana infects all RPW stages under field conditions

Bb203 increases RPW mortality in the Field (canary palm trial)

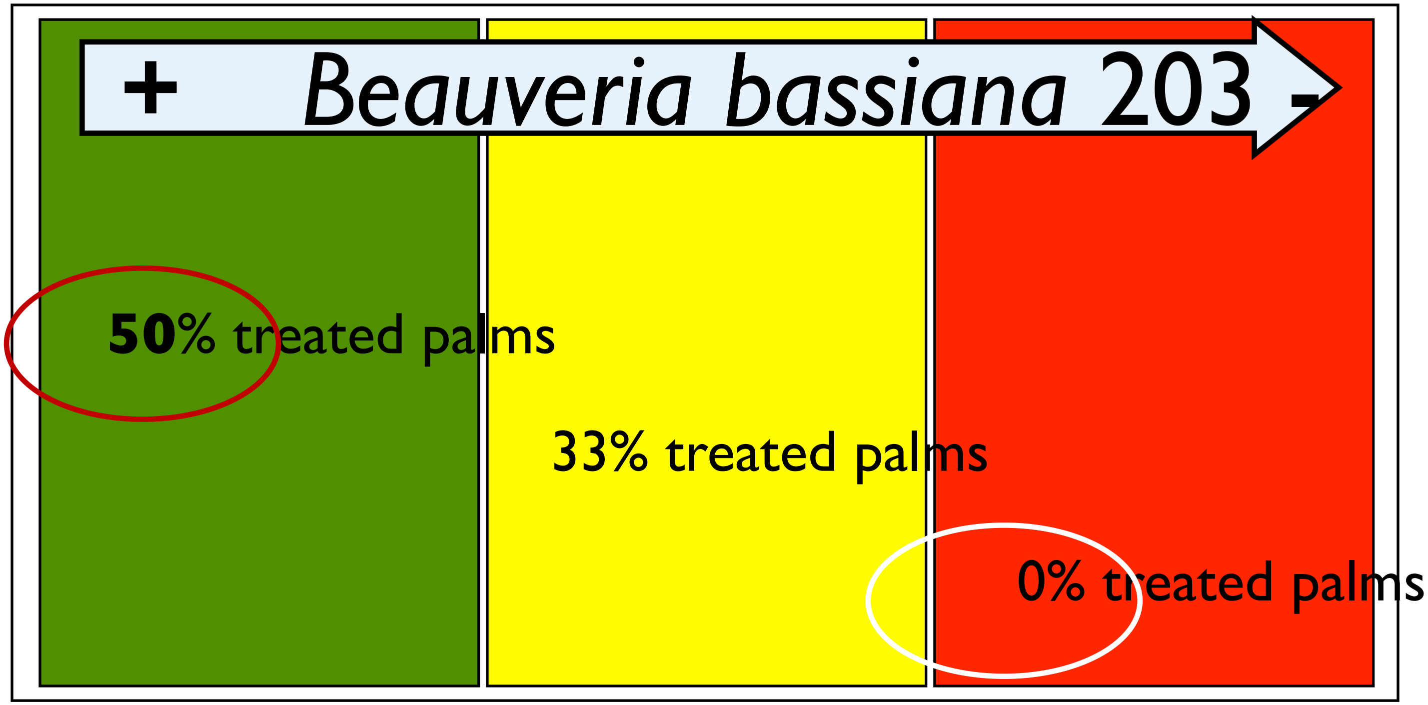




Güerri-Agulló et al. (2011)

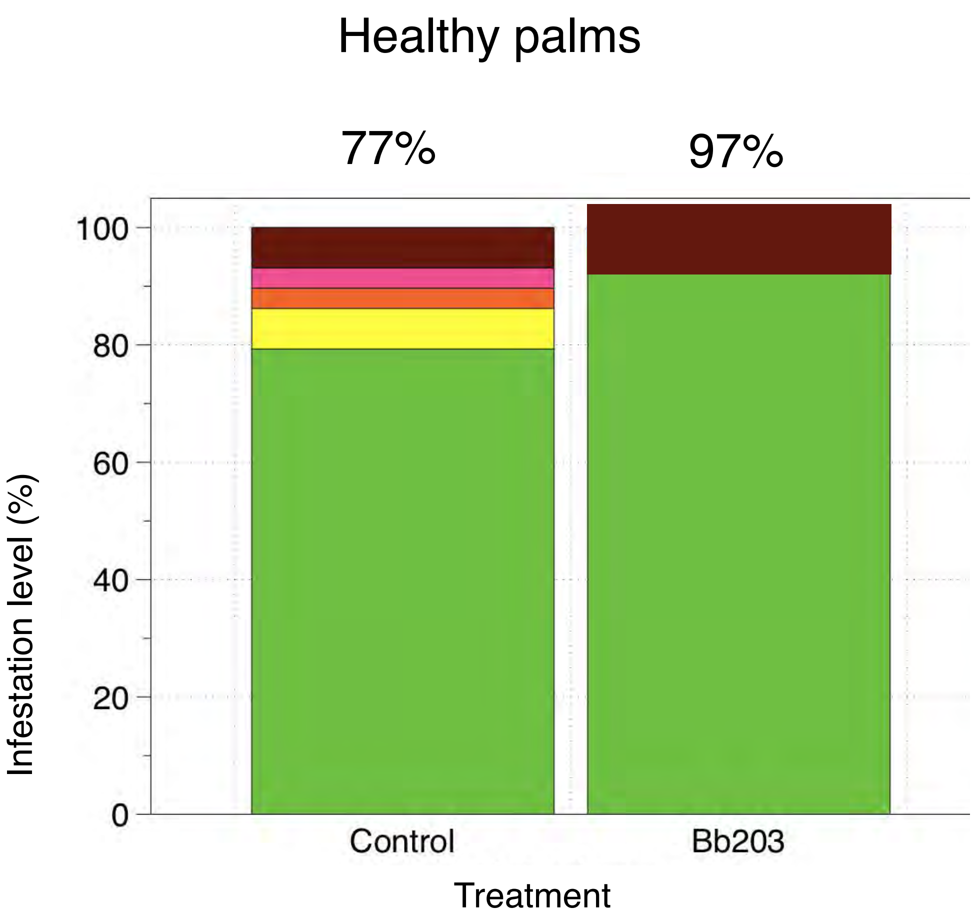
B. bassiana persists in the field

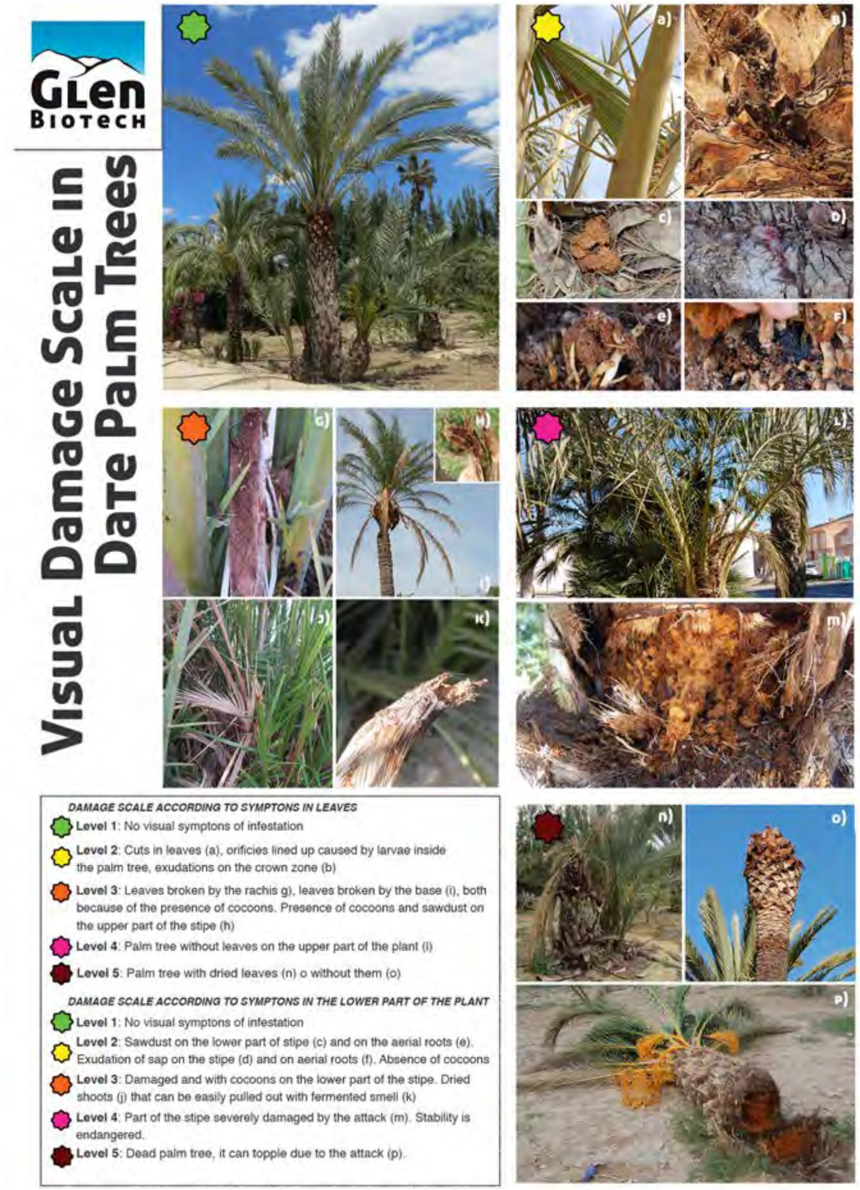
Canary palm trial.
Data collected 12 months after last Bb 203 treatment



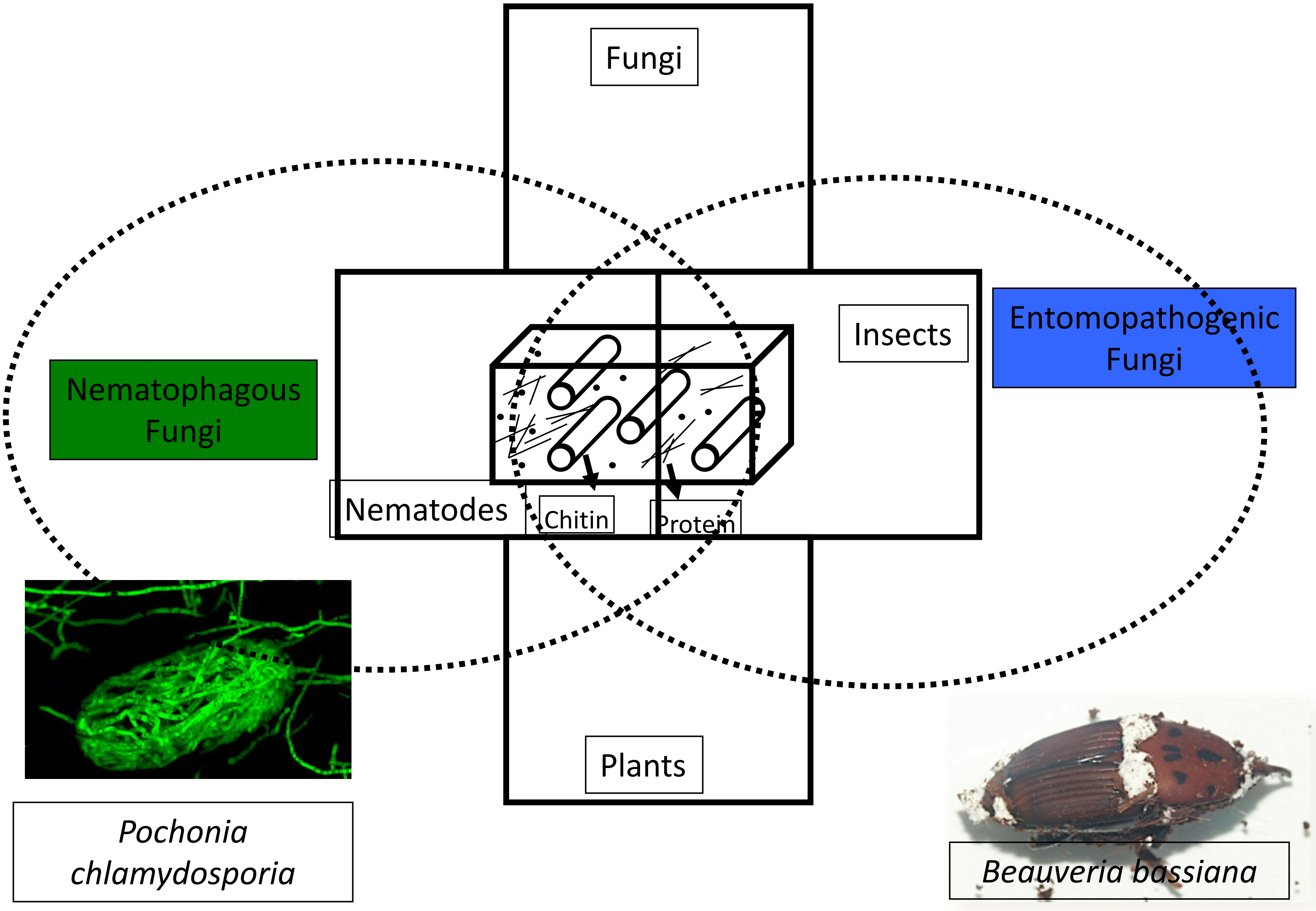
	% Healthy Palms		
Bb203 treated	75	62	-
Non-treated	60	56	38

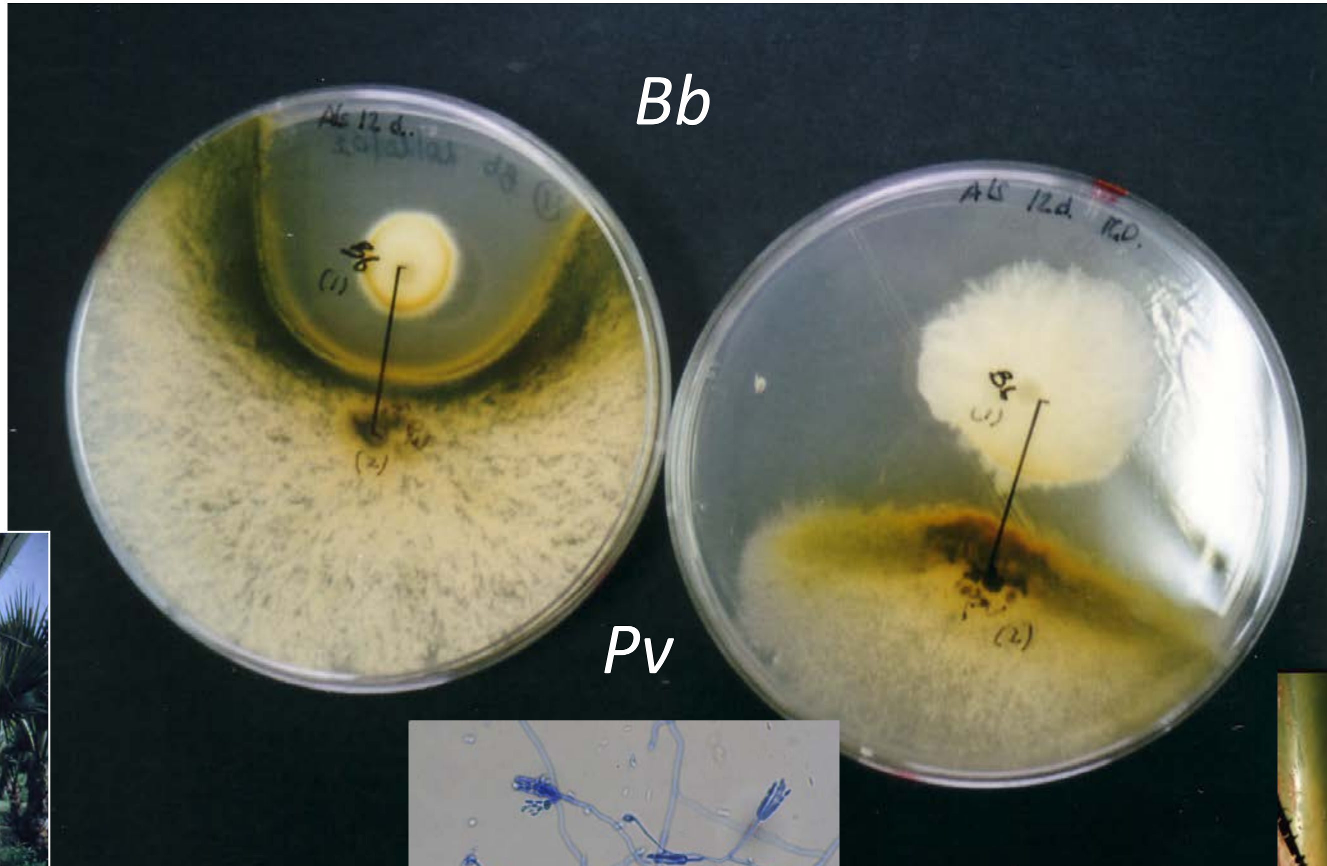
Bb203 reduces RPW palm infestation in the Field (date palm trial)

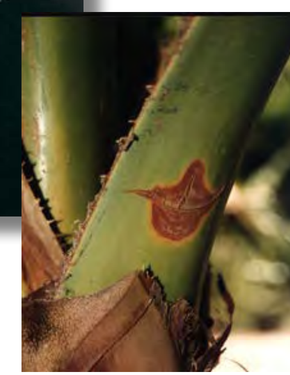




B. bassiana displays a Multitrophic behaviour







B. bassiana (Bb) can inhibit fungal pathogens of palms (*Pv*)

B. bassiana is a Palm Endophyte

B. bassiana is a date palm root endophyte

(Mohamed Mahmoud et al. 2017)

G Model
RIAM 417 1–5
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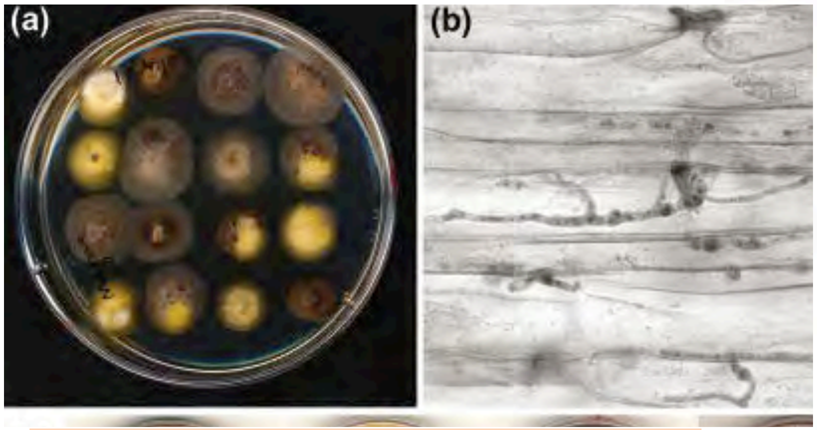
Table 2
Data summary and classification of endophytic fungi colonizing roots of date palms in three dunes of SE Spain into operational taxonomic units (OTUs) and morphological taxa.

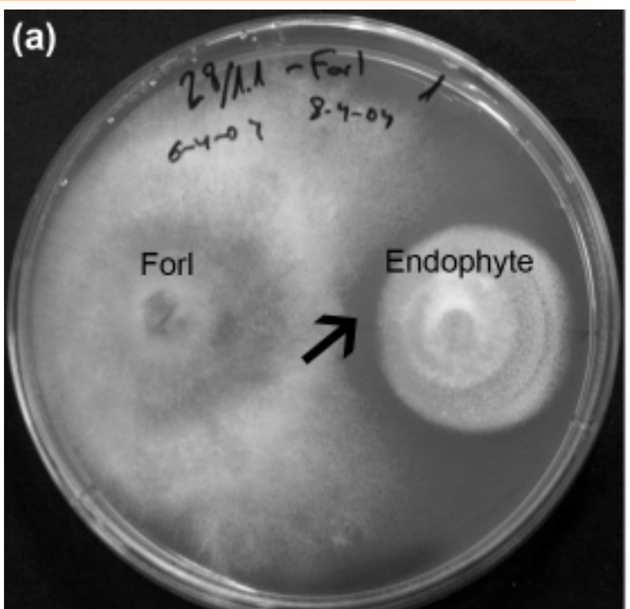
OTU/taxon	Identification methods	Gen Bank accession numbers of sequenced isolates/Site sampling	Closest related species (NBC and BLASTn) Query coverage and identity (%)	Classification and closest related taxa	Number of isolates per sampling site			
					S. Juan	El C.	G.	Sum
OTU 1	Morphological and molecular	KP006352, KP006351, KP006350, KP006348 (San Juan) KP006349, KP006356, KP006357, KP006355 (Guardamar) KP006353, KP006354 (El Carabassi)	*Clonostachys rosea* (KJ158182.1) 574/568 (99%)	A, S, Hypocreales *Clonostachys* sp.	52	18	9	79
OTU 2	Morphological and molecular	KP006338, KP006339, KP006337, KP006336, KP006340, KP006335 (Guardamar)	*Fusarium equiseti* (JQ936180.1) 553/553 (100%)	A, S, Hypocreales *Fusarium* sp.	0	0	11	11
OTU 3	Morphological and molecular	KP006342, KP006341, KP006334, KP006343 (San Juan) KP006333 (El Carabassi)	*Fusarium solani* (KC254048.1) 580/580 (100%)	A, S, Hypocreale *Fusarium* sp.	10	1	0	11
OTU 4	Morphological and molecular	KP006332, KP006331 (El Carabassi)	*Penicillium commune* (KC009833.1) 593/593(100%)	A, E, Eurotiales *Penicillium* sp.	0	6	0	6
OTU 5	Morphological and molecular	KP006346 (Guardamar), KP006368 (El Carabassi)	*Aspergillus tubingensis* (EF621571.1) 607/607 (100%)	A, E, Eurotiales *Aspergillus* sp.	0	1	1	2
OTU 6	Morphological and molecular	KP006359 (San Juan) KP006358 (El Carabassi)	*Beauveria bassiana* (AJ560666.1) 576/576 (100%)	A, S, Hypocreales *Beauveria bassiana*	4	1	0	5
			(AY622994) 290/346 (86%)					
OTU 9	Morphological and molecular	KP006361 (El Carabassi)	*Phomopsis lagerstroemiae* (AY622994) 159/162 (98%)	A, S, Diaporthales *Phomopsis* sp.	0	1	0	1
OTU 10	Morphological and molecular	KP006366 (El Carabassi)	*Corynespora cassiicola* (EU822309.1) 230/244 (94%)	A, D, Pleosporales Pleosporales sp.	0	2	0	2
OTU 11	Morphological and molecular	KP006367 (El Carabassi)	*Ilyonectria radicicola* (AF220968.1) 551/545 (99%)	A, S, Hypocreales *Ilyonectria* sp.	0	9	0	9
OTU 12	Morphological and molecular	KP006369 (San Juan)	*Clonostachys* sp. (AJ880438.1) 131/142 (92%)	A, S, Hypocreales Hypocreales sp.	28	0	0	28
OTU 13	Morphological and molecular	KP006347 (San Juan)	*Aspergillus sclerotiorum* (KC478519.1) 620/626 (99%)	A, E, Eurotiales *Aspergillus* sp.	3	0	0	3
OTU 14	Morphological and molecular	KP006360 (San Juan)	*Phomopsis asparagi* (KC590096.1) 565/581 (97%)	A, S, Diaporthales *Phomopsis* sp.	5	0	0	5
OTU 15	Morphological and molecular	KP006362 (San Juan)	*Marasmiellus candidus* (HM240532.1) 661/717 (92%)	B, Ag, Agaricales Marasmiaceae	6	0	0	6
OTU 16	Morphological and molecular	KP006363 (San Juan)	*Myrothecium verrucaria* (AJ302003.1) 588/588 (100%)	A, S, Hypocreales *Myrothecium* sp.	2	0	0	2
OTU 17	Morphological and molecular	KP006364 (San Juan)	*Diaporthe hongkongensis* (NR111848.1) 583/530 (91%)	A, S, Diaporthales Diaporthales sp.	1	0	0	1
Taxon 1	Morphological	–	–	A, S, Hypocreales Nectriaceae *Fusarium* sp.	94	48	37	179
Taxon 2	Morphological	–	–	A, S, Hypocreales *F. oxysporum*	0	0	5	5
Taxon 3	Morphological	–	–	A, E, Eurotiales *Paecilomyces* sp.	5	3	2	10
Taxon 4	Morphological	–	–	A, E, Eurotiales *Penicillium* sp.	2	3	1	6

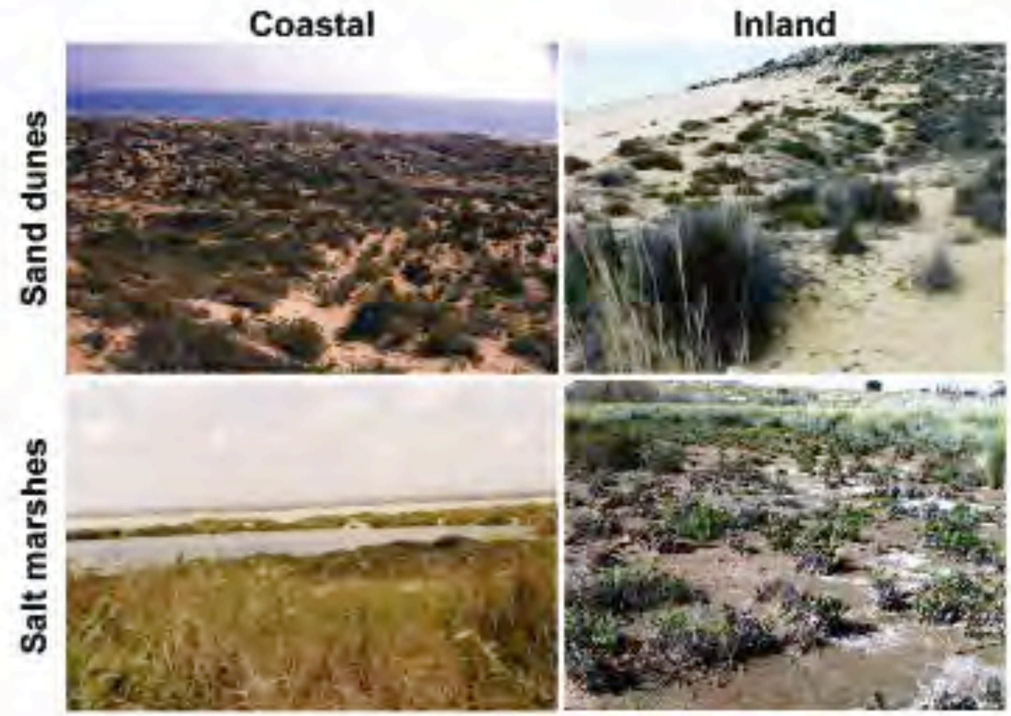
Please cite this article in press as: Mahmoud FM, et al. Endophytic fungi associated with roots of date palm (*Phoenix dactylifera*) in coastal dunes. Rev Iberoam Micol. 2016. http://dx.doi.org/10.1016/j.riam.2016.06.007

Fungal Root Endophytes help Natural vegetation cope with stress



70% Root samples!!!



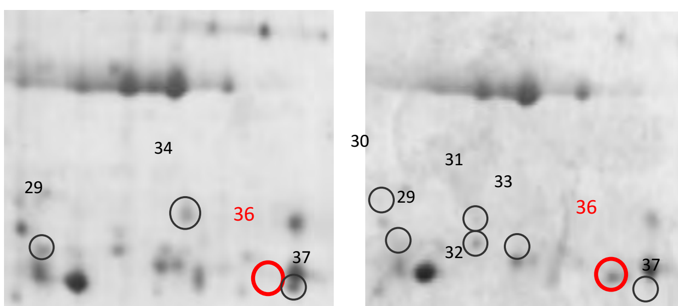


Drought, Salt and Root Pathogens

(Macia-Vicente et al., 2008, 2012)

B. bassiana induces Date Palm defenses

Control



R gene coding Proteins
Antioxidant Proteins
Hydrolytic Enzymes
Heat shock Proteins

Control

B. bassiana

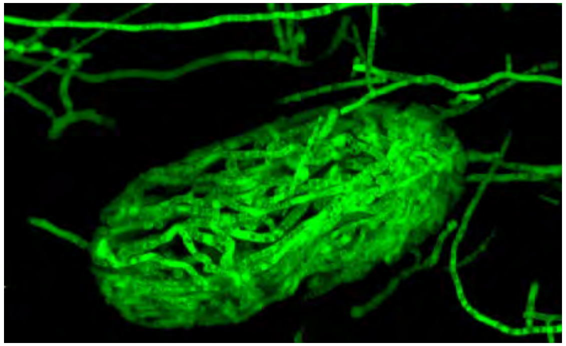
phenolics (tannins)

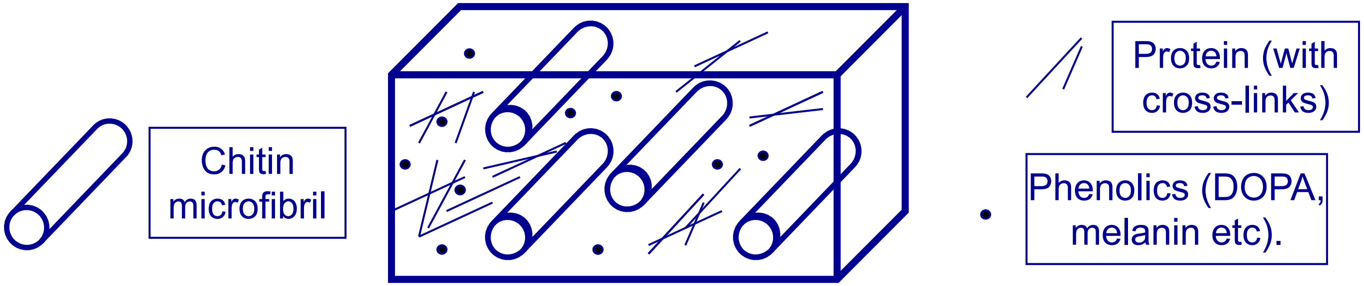
(Gómez-Vidal, *et al*. 2008, 2009).

Chitosan: Biocontrol Fungi enhancer

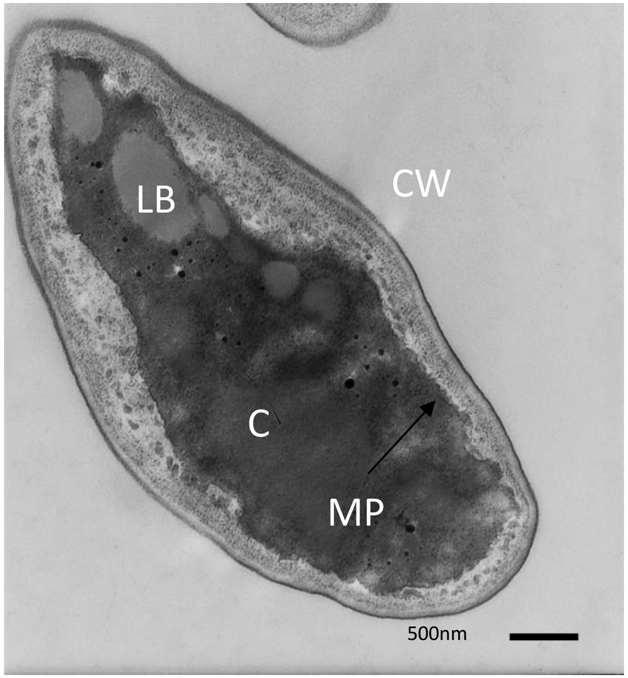
Chitin in Exoskeletons/Cell Walls

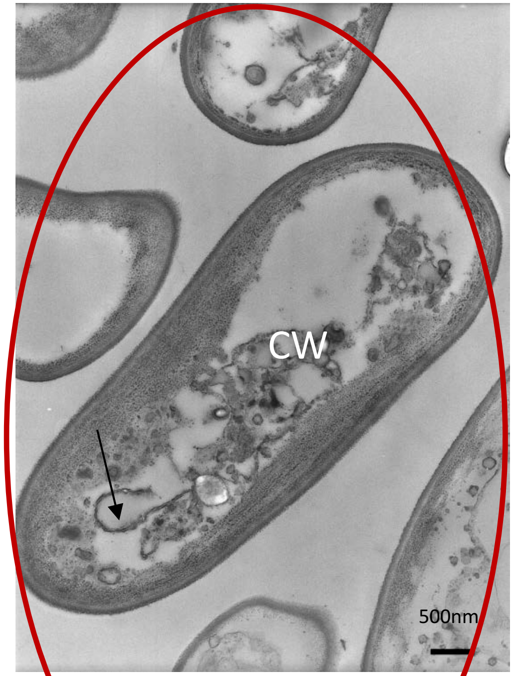
- Chitin main component (flexible/resistant)
- Highly adaptative (Cambric 540 MA)
- Chitin →Chitosan (CDAs)
- Biodegradation (Chitinases/Chitosanases)





Chitosan destroys the plasma membrane of plant pathogenic fungi





CW = cell wall celular

PM =plasma membrane

LB = lipid body

C = cytoplasm

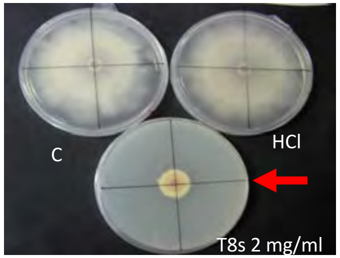
Control (no chitosan)

Chitosan 0.01 mg/ml

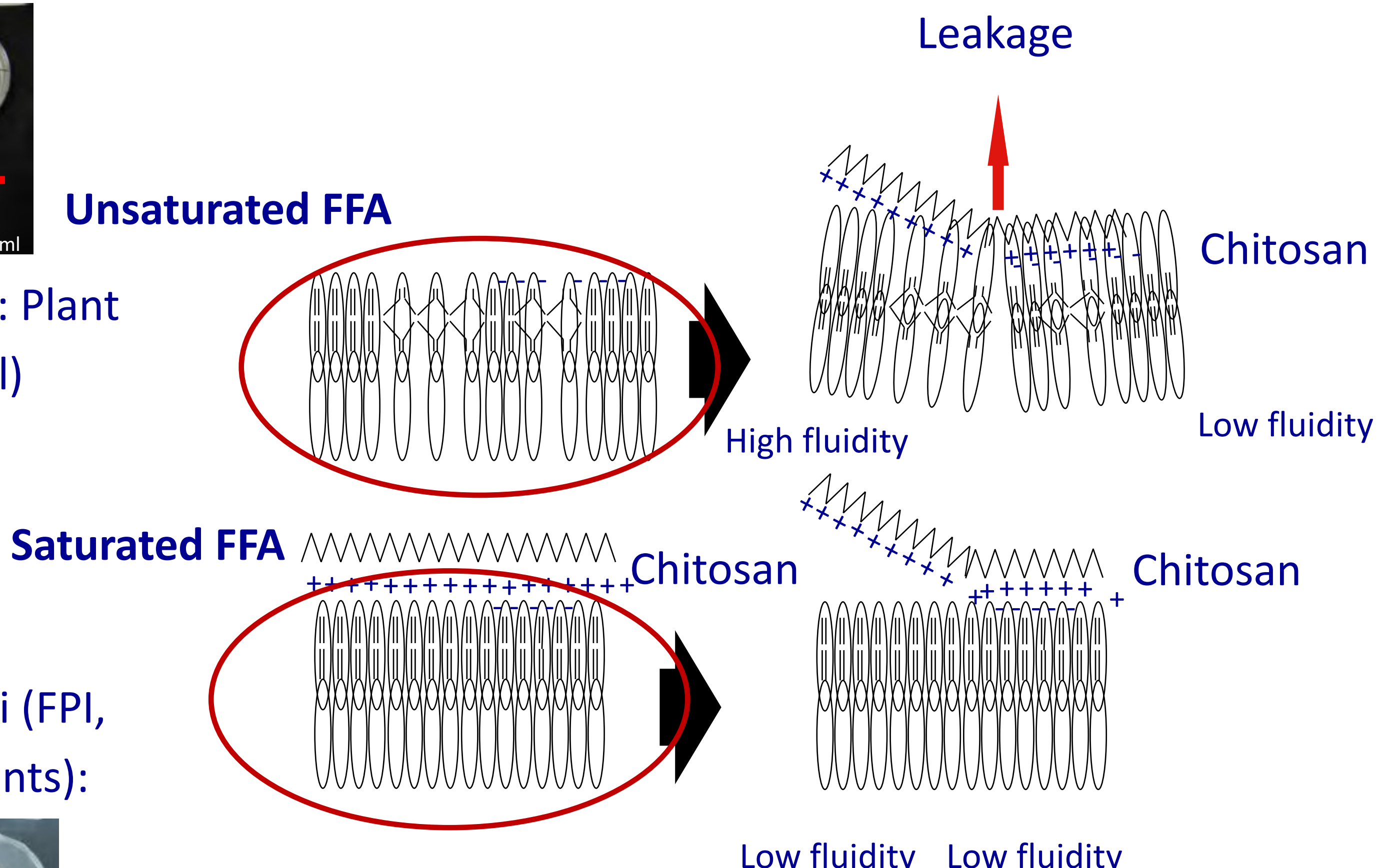
(*Fusarium oxysporum* f.sp. radicis-lycopersici)

Palma-Guerrero et al. (2008)

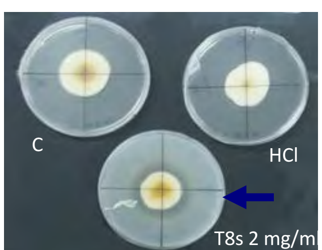
Low-fluidity Membrane makes *B. bassiana* resistant to chitosan



Sensitive Fungi: Plant Pathogens (ForI)

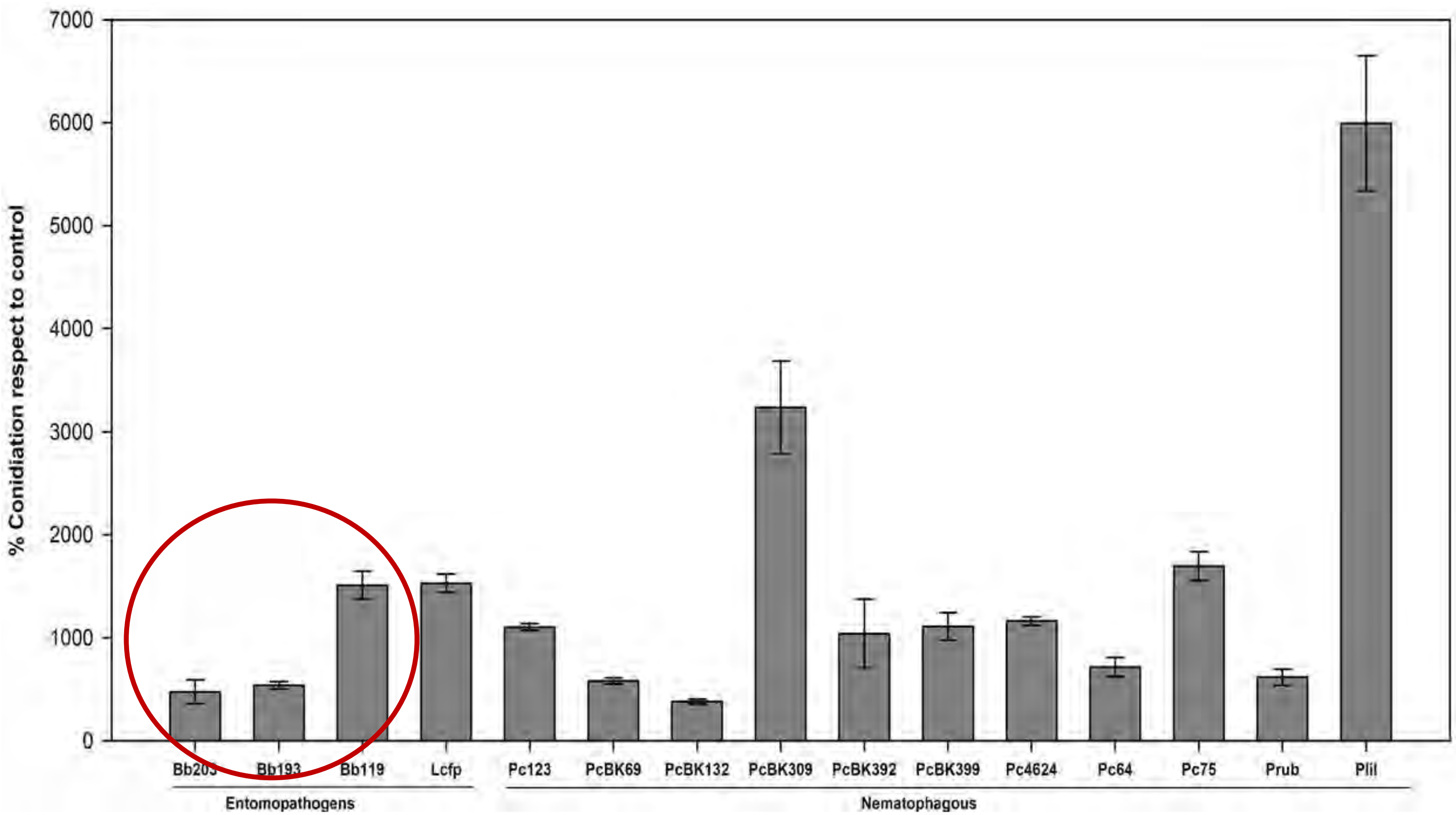


Resistant Fungi (FPI, Biocontrol Agents):



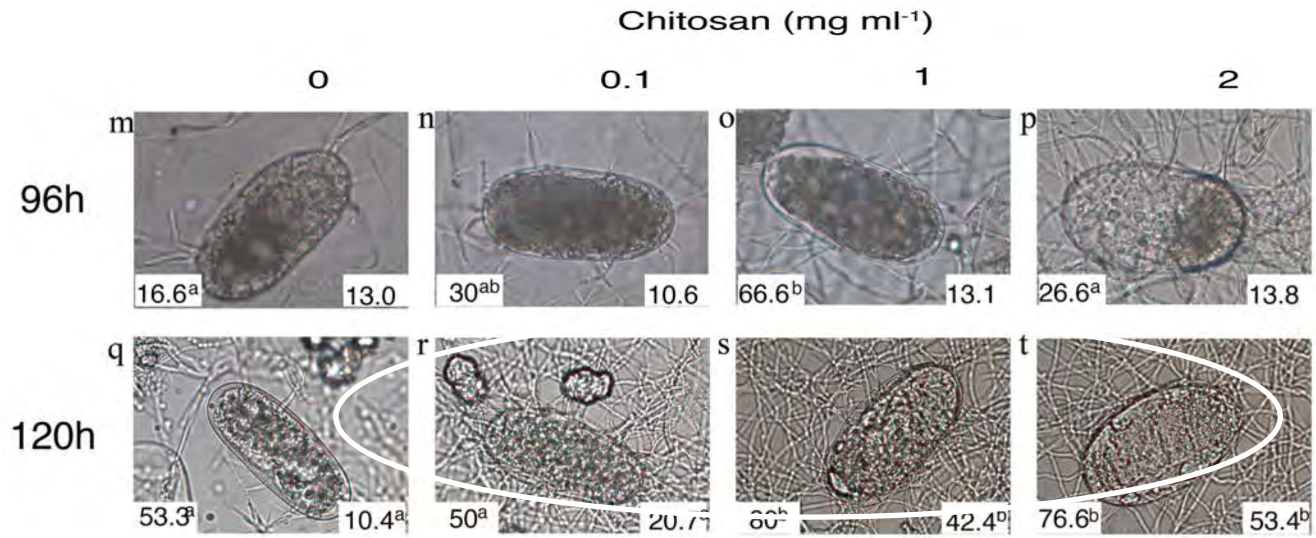
Palma et al., 2010 Molecular Microbiology

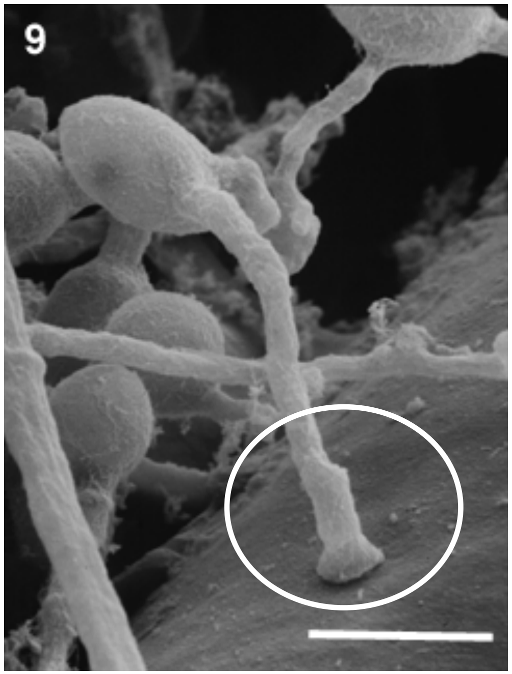
Chitosan stimulates *B. bassiana* sporulation



Palma et al. 2010,
PATENTED

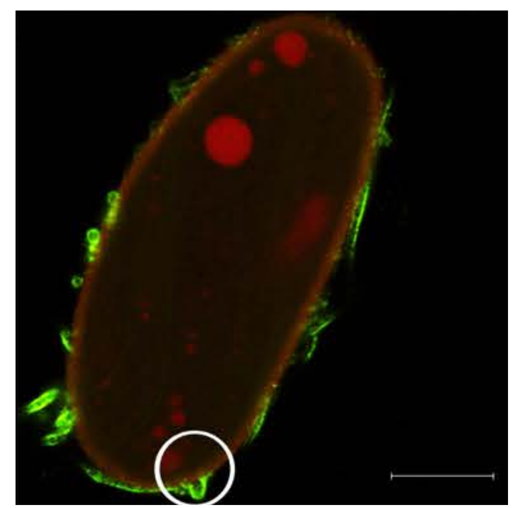
Chitosan enhances virulence of Fungal Parasites of Invertebrates



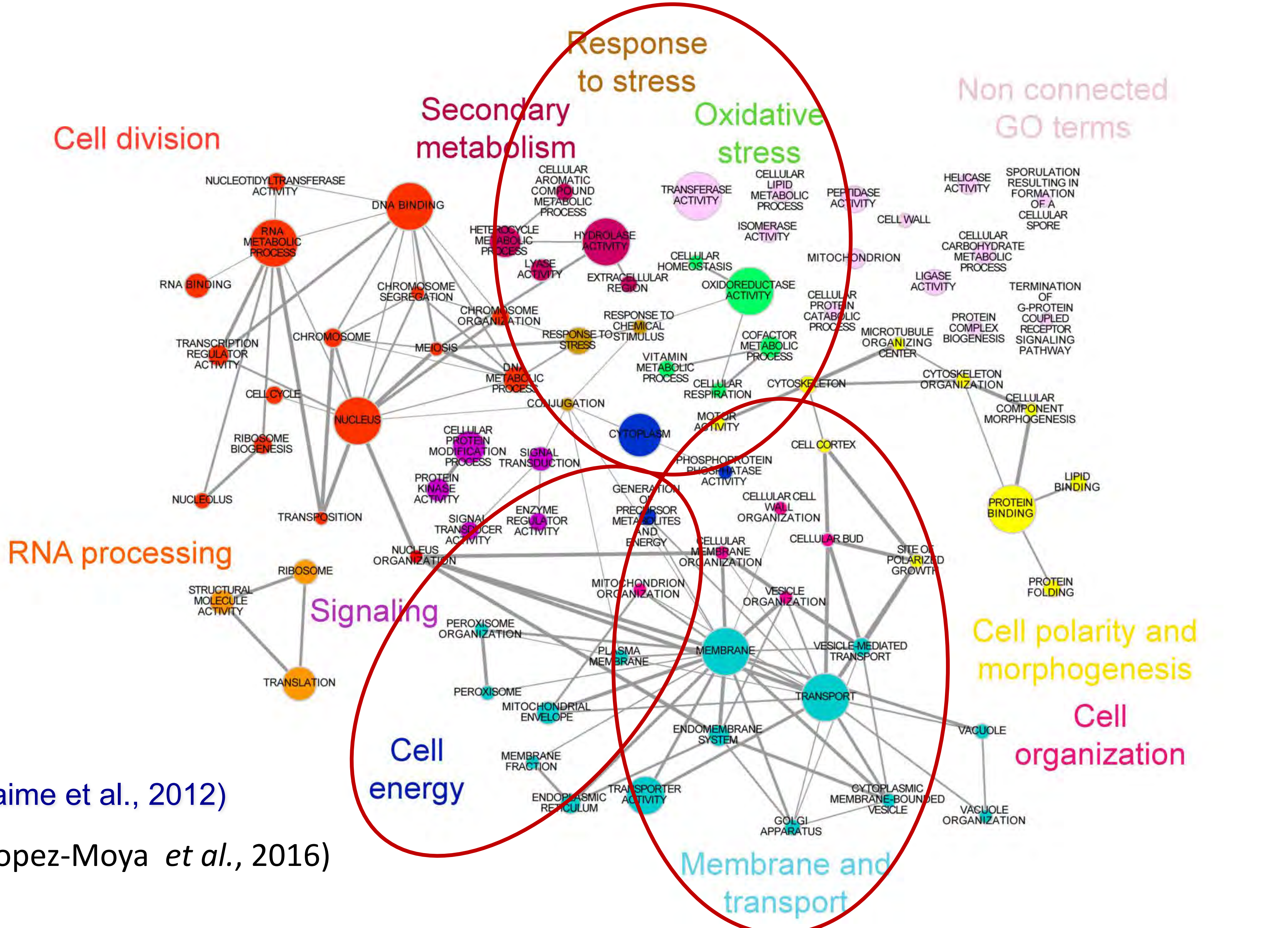


Palma et al., 2010; Escudero *et al.*, 2016)
PATENTED

VcP1/S8 serine protease



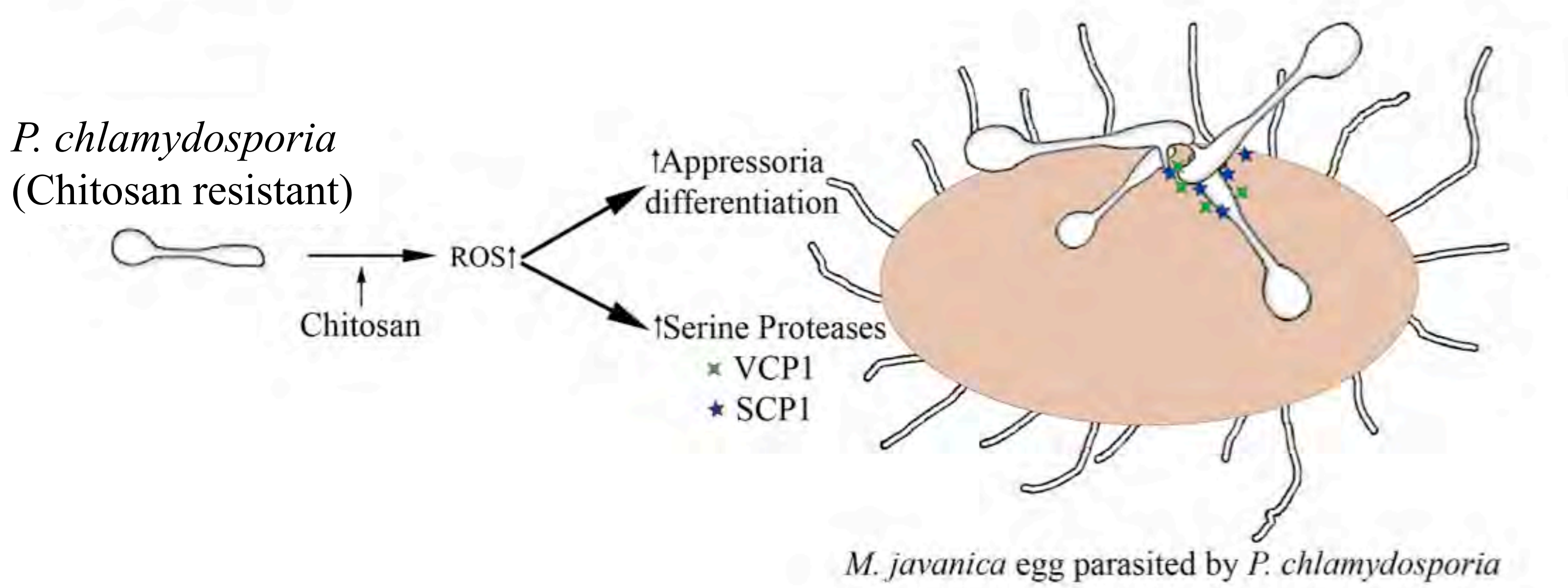
Chitosan activates stress response genes in fungi



(Jaime et al., 2012)

(Lopez-Moya *et al.*, 2016)

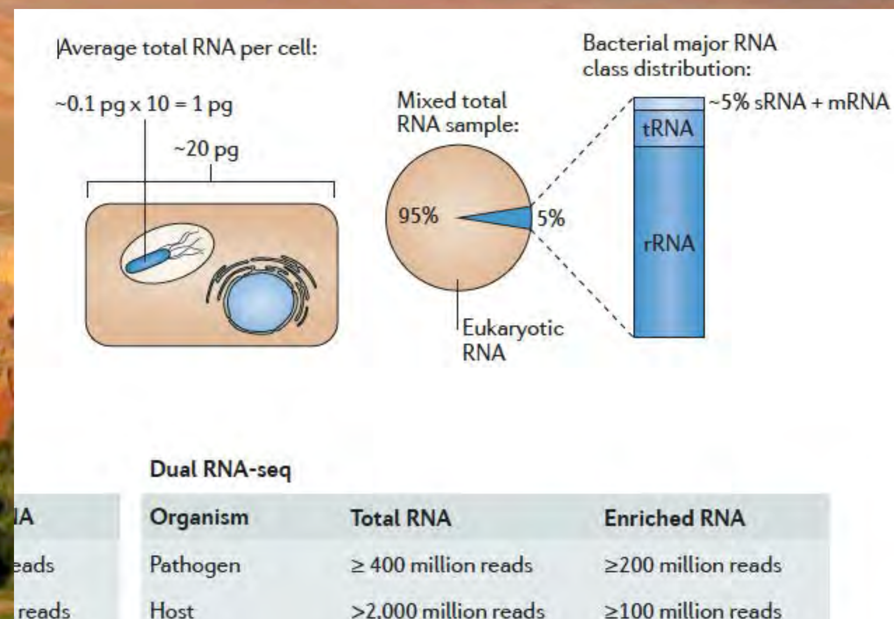
Model: Chitosan enhaces ROS→Promotes Appresoria differentiation and Protease expression in Biocontrol Fungi (Escudero et al. 2016)



NEW STRATEGY:

Palm/Antagonist (eg. *B. bassiana*)/RPW/*Chitosan*

-OMICS (RNAseq/Proteomics/Metabolomics)



Organism	Total RNA	Enriched RNA
Pathogen	≥ 400 million reads	≥200 million reads
Host	>2,000 million reads	≥100 million reads

ALL GENOMES AVAILABLE

DISCOVERY OF NEW TOOLS (Proteins/Metabolites) FOR PALM STRESS MANAGEMENT

Lopez-Moya et al., 2016
Westermann et al., 2012

Conclusions

1. *B. bassiana* can manage sustainably RPW
2. *B. bassiana* can induce palm defences
3. Chitosan enhances performance of *B. bassiana*
4. -*omics* can improve Palm stress management

THANKS FOR YOUR ATTENTION!!!


THANKS TO THE PLANT PATHOLOGY LAB
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