



Sustainability, Application and Delivery of Biological Control Agents

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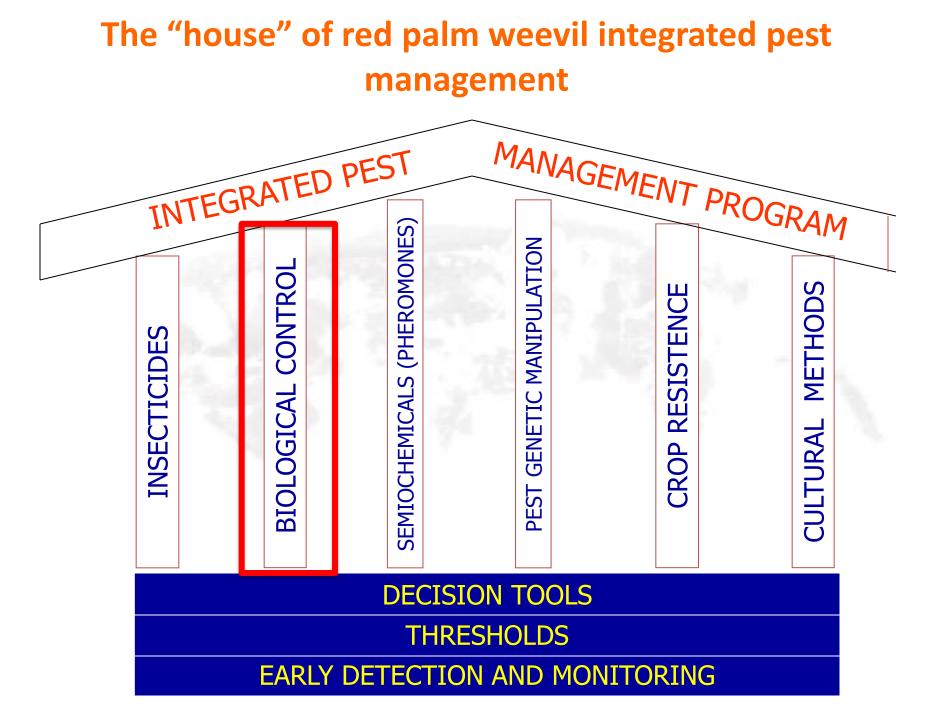
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Information Note

Scientific Consultation and High-Level Meeting on Red Palm Weevil Management

29-31 March, 2017

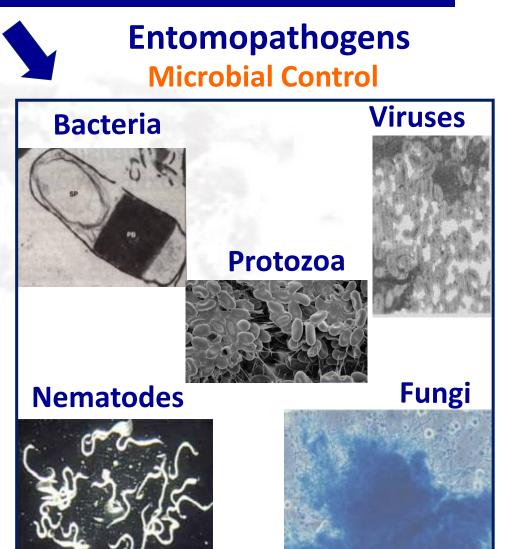
FAO, Rome, Italy



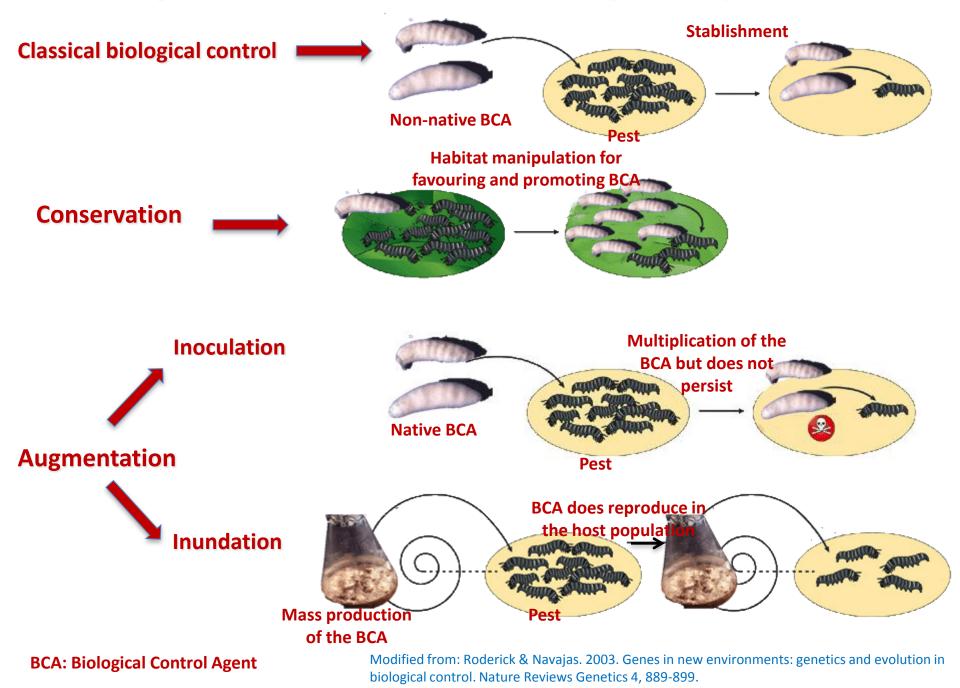
Biological control is defined as the reduction of pest populations by using other organism, sometimes their own natural enemies (Van Lenteren, 2012)







Strategies for the use of microbial control agents for pest control



Biological control is defined as the reduction of pest populations by using other organism, sometimes their own natural enemies (Van Lenteren, 2012)



Macrobial Control



Ortega-García et al. 2016. Natural Enemies of *Rhynchophorus ferrugineus* and *Paysandisia archon*. Chapter 8. In: Handbook of Major Palm Pests: Biology and Management (Victoria Soroker and Stefano Colazza eds.). Wiley.

Red palm weevil control by Predators

- Predators kill and eat many insects to complete their life-cycle (i.e. mammals, birds, amphibians, reptiles, fishes and insects.....)
- The earwigs Chelisoches morio (Fabricius) (Dermaptera: Chelisochidae) and Euborellia annulipes (Lucas) (Dermaptera: Anisolabididae) haven been described as common predators of *R. ferrugineus* eggs and larvae in crowns of different palm species around the world
- Mites from the family Uropodidae (Acari: Mesostigmata) have also been observed in large numbers in association with red palm weevil in Israel



- These mites are not considered to play a role as biological control agents
- However, as they populate on each individual in huge numbers it is expected that they should significantly compromise red palm weevil flight abilities and thus the dispersion

Red palm weevil control by Parasitoids

- Parasitoids are arthropods whose immature stages develop on or inside their host, causing its death, while adults are free living
- The host-range of parasitoids is usually limited to one or few closely related species because their life-cycles must be very fine tuned
- The ectoparasitoid Scolia erratica Smith (Hymenoptera: Scoliidae) has been described naturally parasitizing red palm weevil larvae in several occasions, although it is not specific of the Curculionidae family
- 40-50% parasitism levels have been reported for the parasitic fly
 Billaea menezesi (Townsend) (Diptera: Tachinidae) in
 Rhynchophorus palmarum in palm orchards in Brazil

Ortega-García et al. 2016

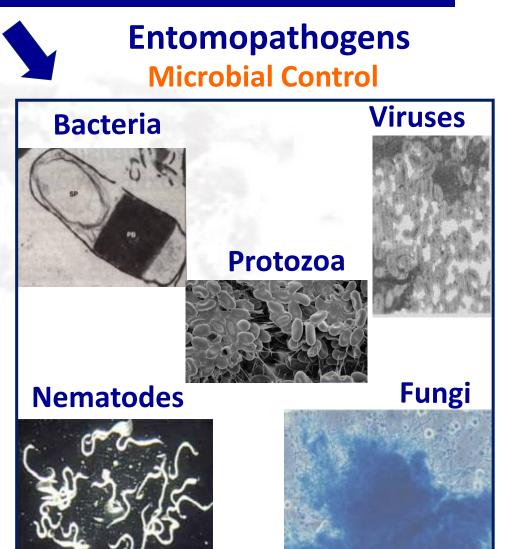
It could be concluded that predators and parasitoids do not shown potential for RPW control apart from its natural activity that should be conserved



Biological control is defined as the reduction of pest populations by using other organism, sometimes their own natural enemies (Van Lenteren, 2012)

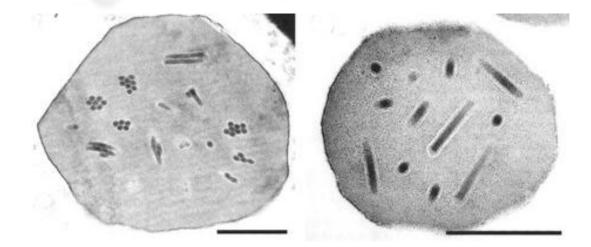






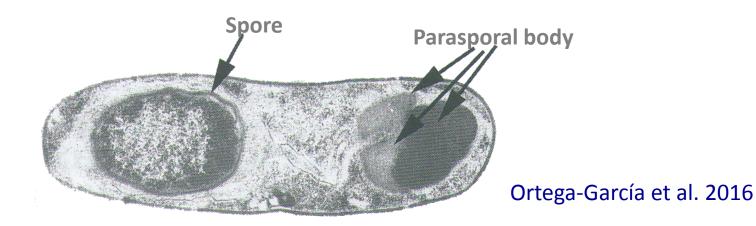
Viruses

- Insect viruses are obligate pathogens that can only reproduce within a host insect. They have to be ingested by the insect host to start the infection process
- First case of a cytoplasmic polyhedrosis virus on RPW in India causing deformed adults and reducing their lifespan (Gopinadhan et al. (1990)
- Viruses have little use as biological control agents (BCAs) against RPW, except a report on their combination with nematodes (Salama and Abd-Elgawad, 2002)



Bacteria

- Entomopathogenic (entomotoxic) bacteria have to be ingested by the insect host to start the infection process
- Several pathogenic bacteria isolated from the red palm weevil have proved successful in laboratory conditions
- Among them, Bacillus sphaericus Neide has shown the highest activity, with low activities of Bacillus thuringiensis Berliner (Salama et al 2004; Manachini et al., 2009; 2011)



Nematodes

- Steinernematidae and Heterorhabditidae families are the most interesting biocontrol species (among the 23 described) carrying specific pathogenic bacteria Xenorhabdus and Photorhabdus
- These nematodes can actively find, penetrate, and infect the insect with their symbiotic pathogenic bacteria which are released into the insect hemocoel
- Natural infections of RPW by Heterorhabditis and Steinernema have been described
- Several tests conducted both in laboratory and field have evaluated the efficacy of EPNs

Xenorhabdus spp.

Photorhabdus spp.

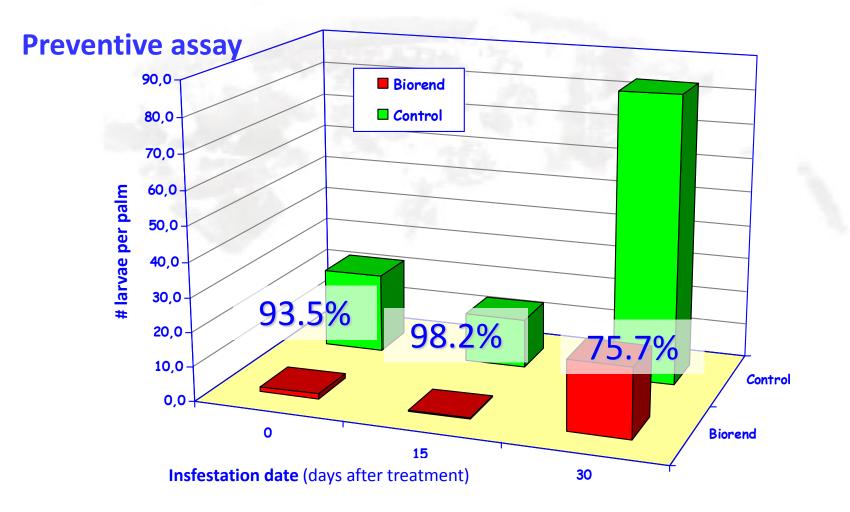
Ortega-García et al. 2016

Nematodes

- Steinernema feltiae Filipjev and Heterorhabditis bacteriophora Poinar have shown high infectivity to all developmental stages of R. ferrugineus in laboratory assays
- These nematodes have been also applied to red palm weevilinfested Canary Islands date palms, where they have been observed actively moving into the tree inner tissues, infecting larvae and adults
- However, in general, inconsistent results have been reported when EPNs are applied in field experiments due to high temperatures, high UV radiation, and low relative humidity that affect nematode survival

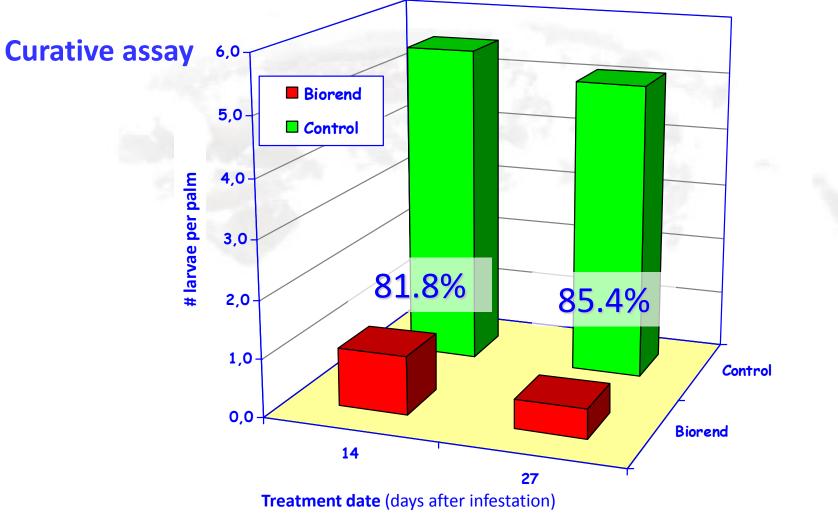
Red palm weevil control by entomopathogenic microorganisms Nematodes + chitosan

Llácer *et al.* (2009). Evaluation of the efficacy of *Steinernema carpocapsae* in a chitosan formulation against the red palm weevil in *Phoenix canariensis*. BioControl 54, 559–565 (doi: 10.1007/s10526-008-9208-3)



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Nematodes + chitosan+imidacloprid)

Dembilio *et al.* (2010). Field efficacy of imidacloprid and *S.carpocapsae* in a chitosan formulation against the red palm weevil in *P. canariensis*. Pest Manag. Sci. 66, 365-370 (doi: 10.1002/ps1882)

Field assay in a nursery (6-8 yr-old *P. canariensis*); 6 treatments:

Control

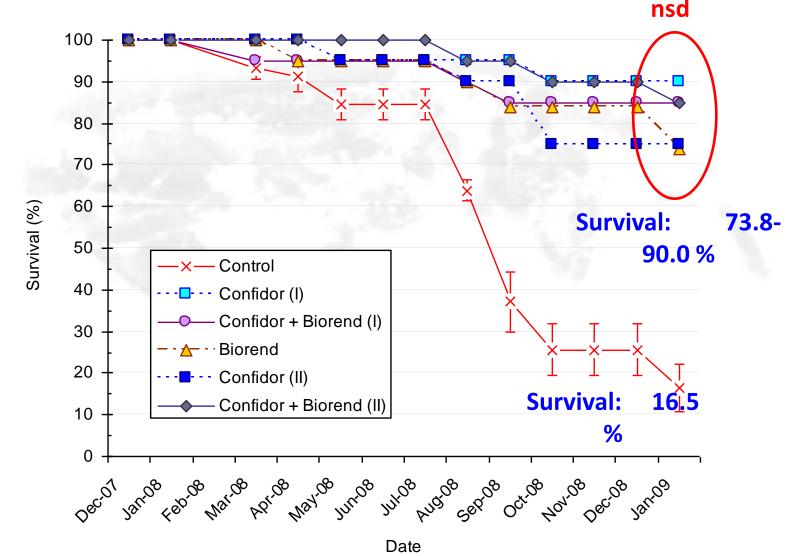
Biorend monthly (S. carpocapsae + chitosan)

Confidor (imidacloprid) drench in either: March and May May and July.

Same as before + Biorend at 1st treatment and September

Red palm weevil control by entomopathogenic microorganisms Nematodes + chitosan+ imidacloprid

Dembilio *et al.* (2010). Field efficacy of imidacloprid and *S.carpocapsae* in a chitosan formulation against the red palm weevil in *P. canariensis*. Pest Manag. Sci. 66, 365-370 (doi: 10.1002/ps1882)



- Therefore, the use of chitosan in the formulation has showed to protect entomopathogenic nematodes at field conditions by increasing and stabilizing their efficacy, which reached 98 and 80 % in preventive and curative assays, respectively (Llácer et al., 2009; Dembilio, et al. 2010)
- Compatible with insecticides (i.e. imidacloprid)
- The key concern with these nematodes is the lack of reproduction of some species inside red palm weevils which limits their commercial exploitation (Mazza et al., 2014) as frequent applications are still needed



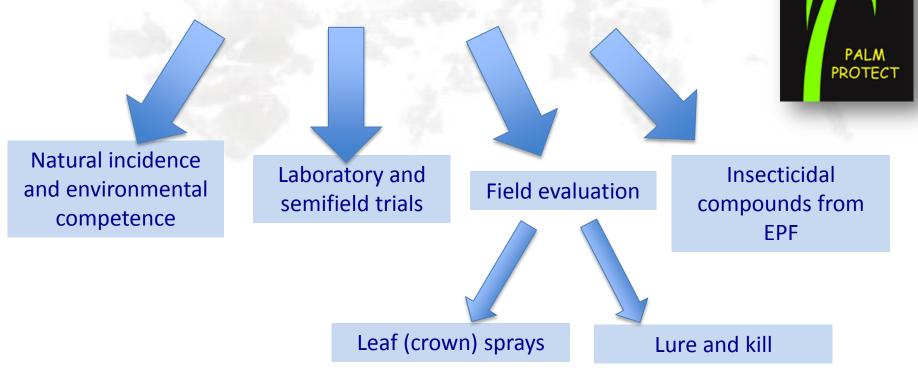






Project KBBE-2011-5-289566. Strategies for the eradication and containment of the invasive pests *Rhynchophorus ferrugineus* Olivier and *Paysandisia archon* Burmeister (2011-2015) PALM PROTECT

Entomopathogenic fungi for RPW control



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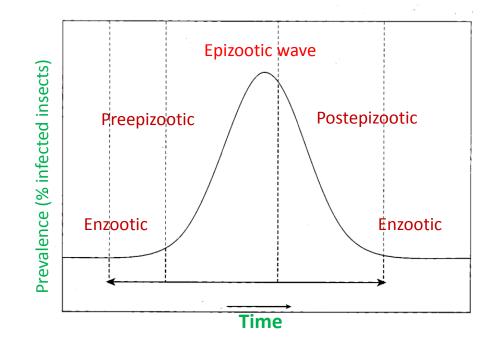
Entomopathogenic fungi for RPW control

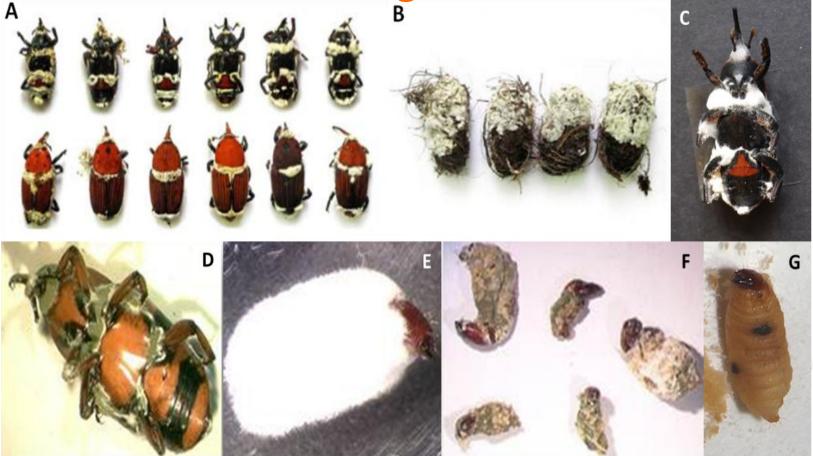
Natural incidence and environmental competence

Project KBBE-2011-5-289566. Strategies for the eradication and containment of the invasive pests *Rhynchophorus ferrugineus* Olivier and *Paysandisia archon* Burmeister (2011-2015) PALM PROTECT



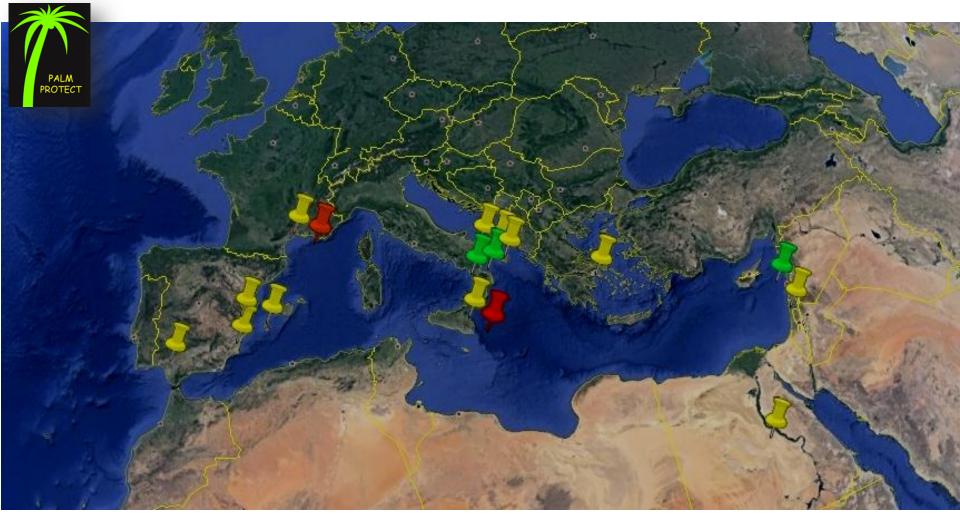
One of the main findings of PALM PROTECT regarding microbial control of RPW is that entomopathogenic mitosporic ascomycetes are the main natural regulators of RPW populations in the Mediterranean basin, with several reports of natural infections close to epizootic levels



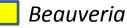


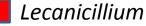
A) Natural epizootic of *Beauveria* and *Metarhizium* spp. in RPW population in Israel. Adults infected with *Metarhizium* spp. ventral and dorsal views. B) Cocoons infected with *Beauveria* spp. C, D) Adults and E, F) larvae infected with *Beauveria* and *Metarhizium* spp. respectively. G) Entry points due to fungal invasion.

24 fungal isolates from diverse locations of the Mediterranean Basin



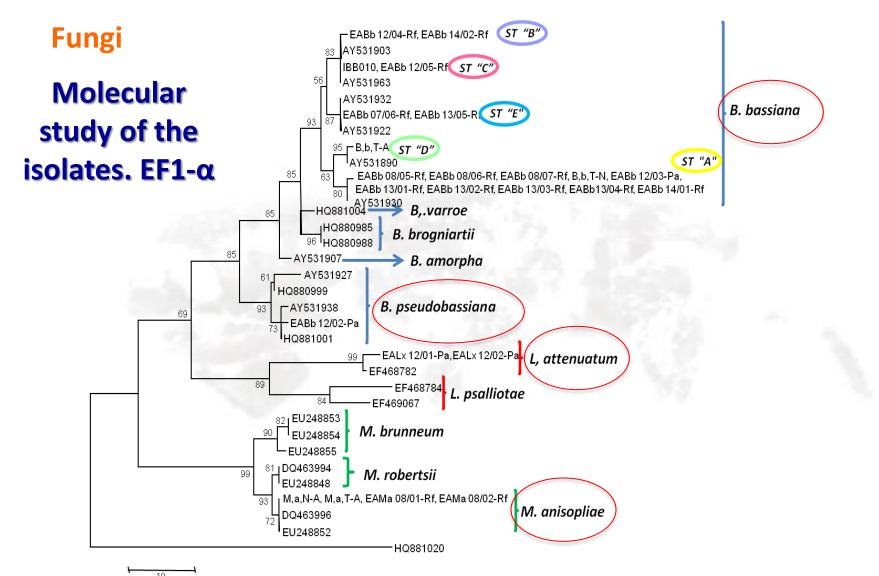




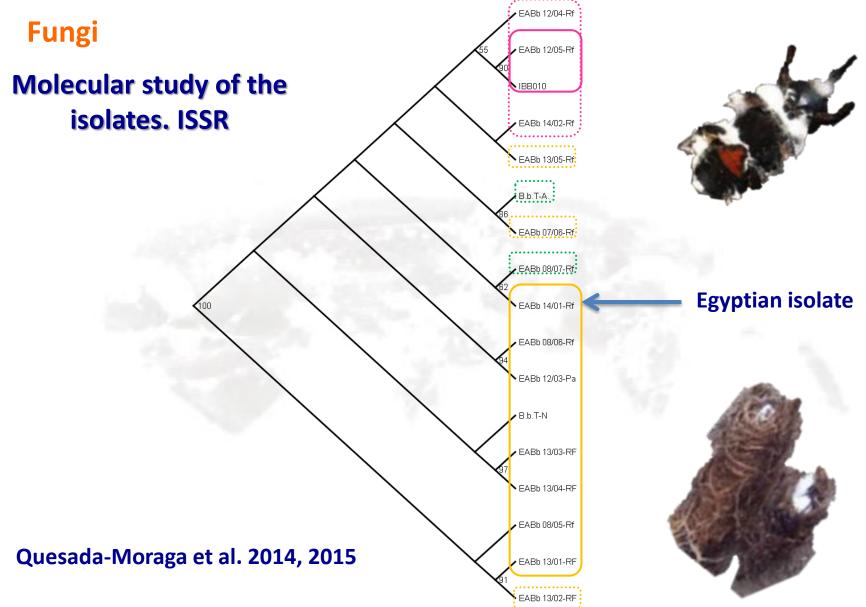




- **Molecular study of the isolates:**
 - Elongation factor (EF1-α)
 - Combined analysis (EF1-α–BLOC)
- > ISSR
- Effect of humidity, temperature, and UV
- Pathogenicity and virulence against *R. ferrugineus* pupae and adults



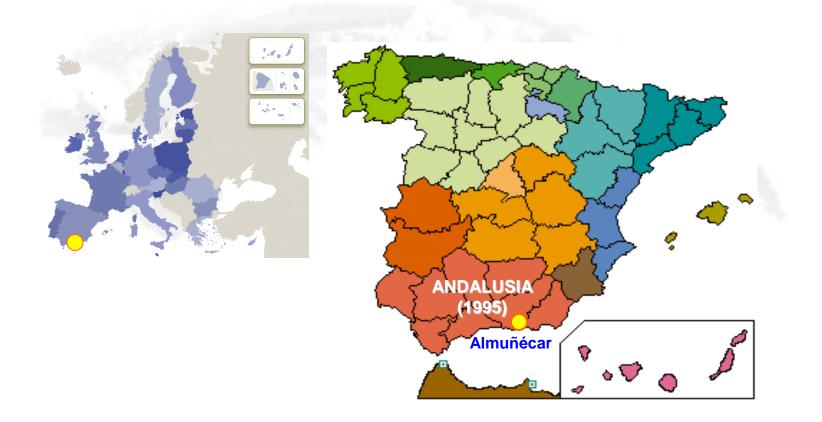
Phylogenetic analysis of entomopathogenic fungi isolates obtained from *R. ferrugineus* and *P. archon* inferred from Maximum Parsimony analysis of EF1- α gene sequences. Bootstrap values (based on 500 replicates) when above 50% are indicated in the branches. Species clades are indicated by vertical bars. Type sequences of *B. bassiana* are defined with the letters "A", "B", "C", "D", and "E", marked in yellow, purple, pink, blue and green respectively.



Phylogenetic tree of genetic relationship between the *B. bassiana* isolates obtained from *R. ferrugeneus* and *P. archon* with base on ISSR makers. Same color and continuous line indicate the same type sequence in the EF1- α and BLOC analysis. Same color and discontinuous line indicate same clade in the EF1- α and BLOC phylogenetic tree.

This diversity and phylogeny tree can be explained only by considering that the original RPW specimens from Egypt reaching Europe (Spain) were probably infested by *B. bassiana*, and also specimens of later invasions

In 1995 *R. ferrugineus* is first detected in the EU: Almuñécar (Andalusia, Spain) very probably coming from palms from Egypt



Rapid spread starting in 2004

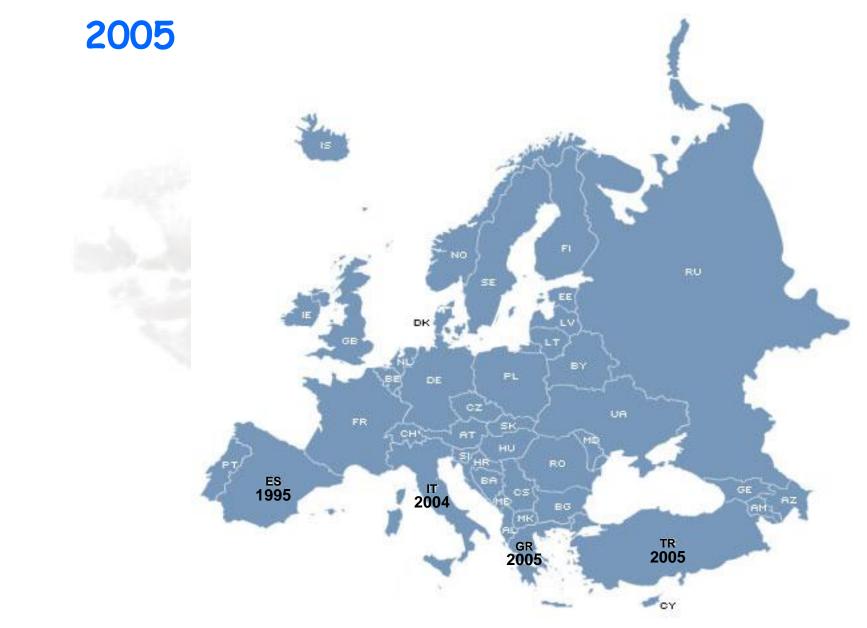


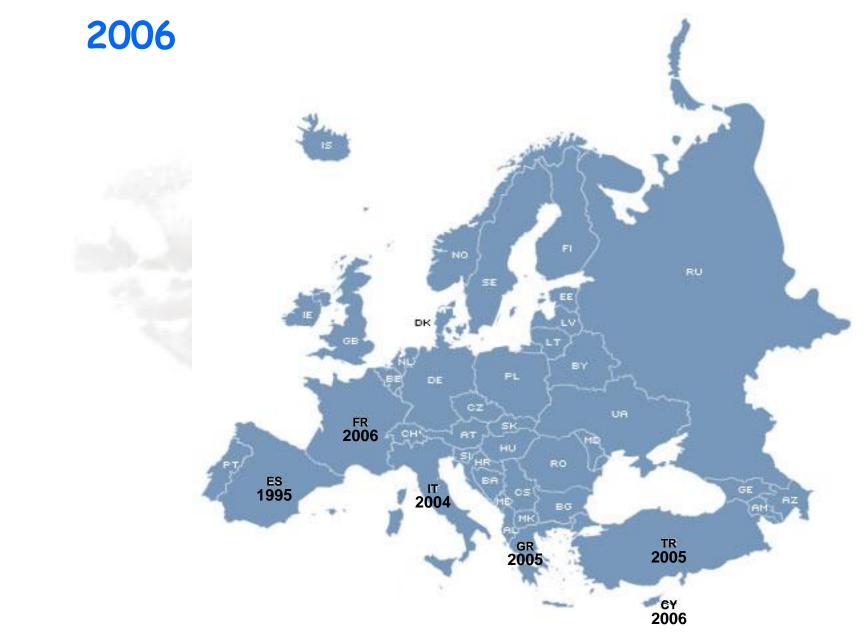


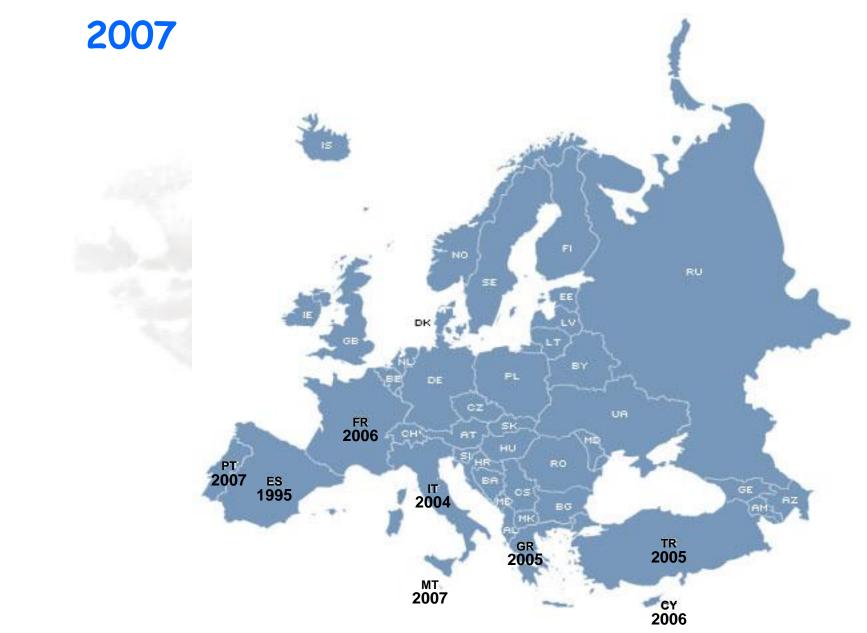
First detection of *B. bassiana naturally infecting R. ferrugineus* pupae in 2007 (Dembilio *et al.,* 2010)

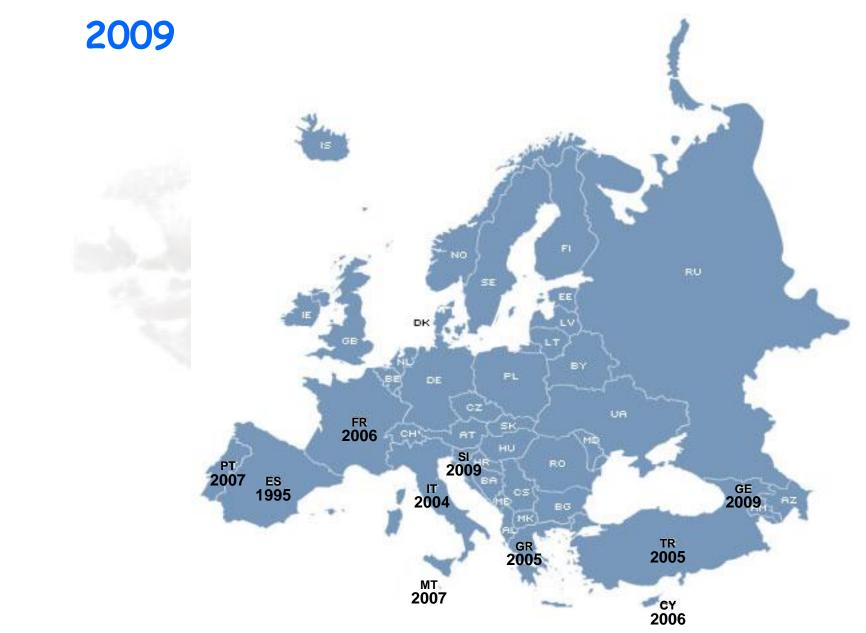
From 1995 to 2007 original *B. bassiana* genotypes evolved in Spain.....and them.....

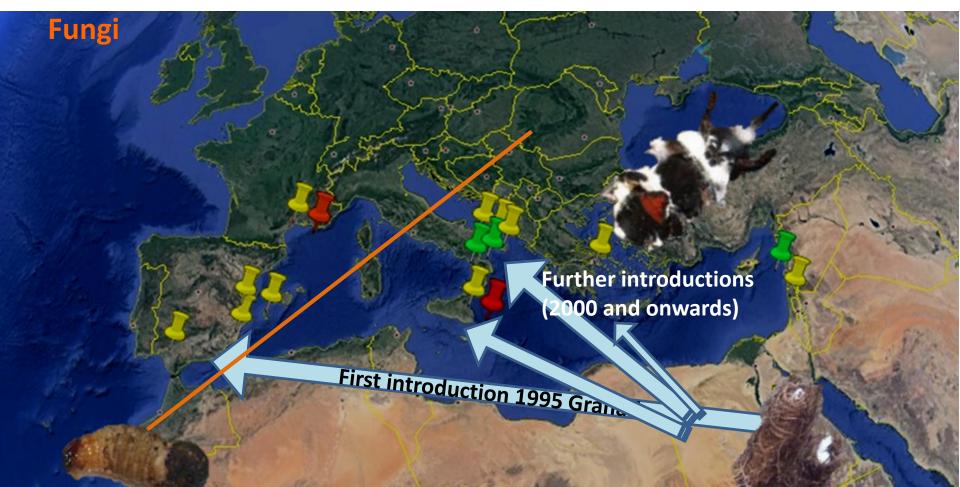












Most of the isolates obtained in Spain and Southern France are evolutionarily very close, while they are further away from the isolates found in southern Italy, Greece and Israel, which are closer to the posible original Egyptian isolates

Fungi

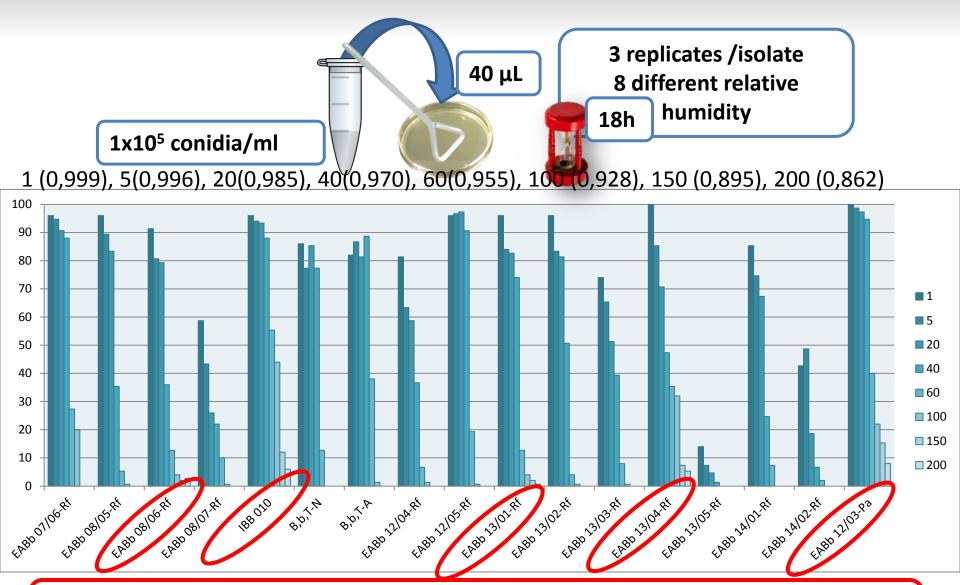
This could support a host-mediated spread of **B.** bassiana from the R. ferrugineus specimens firstly introduced into Europe from Egypt with geographic (introduction point) and time (since introduction) factors determining evolution patterns in the invaded areas. The other palm invasive species, P. archon, could also have played an important role in this host-mediated spread.



- Molecular study of the isolates:
 - **Elongation factor (EF1-α)**
 - **Combined analysis (EF1-α–BLOC)**
 - > ISSR
- Environmental competence (RH, Tª, UV-B)
- Pathogenicity and virulence against *R. ferrugineus* pupae and adults

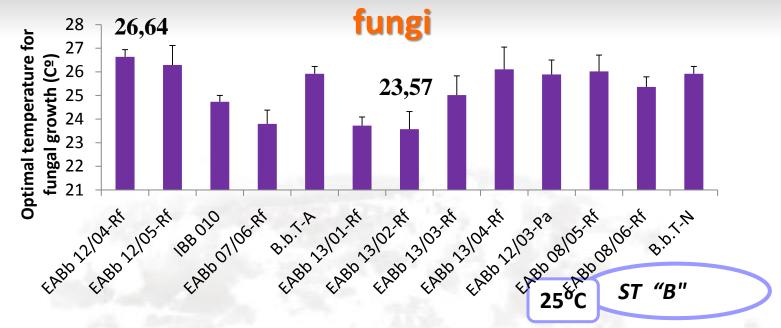
Quesada-Moraga et al. 2014, 2015

Effect of relative humidity (MPa) on conidial germination



Only five isolates, four of them from the same type sequence, showed germination even at 0,862 aw (200MPa). This fact should be considered in future strain selection.

Effect of temperature on in vitro radial colony growth of

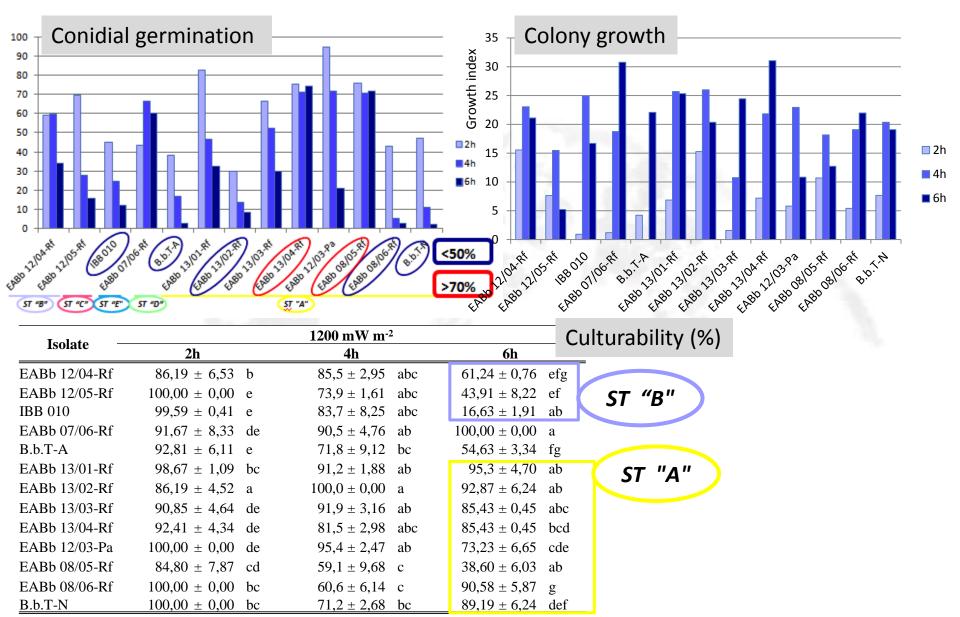


Conidial germination (%) of Beauveria bassiana isolates at different temperatures

	Isolate	Temperature (°C)								
		15	15		20		5		30	
	EABb 12/04-Rf	$25,\!69 \pm 0,\!75$	bcd	69,333 ±	1,764bc	98	± 2a	$86,667 \pm$	3,712ab	
	EABb 12/05-Rf	$18,07 \pm 1,06$	de	52,667 \pm	6,36cd	82,667	± 1,333a	$67,333 \pm$	4,055bc	C
	IBB 010	$21,83 \pm 1,89$	cd	54,667 \pm	1,764cd	82	± 4,163a	70,667 \pm	4,372abc	r
	EABb 07/06-Rf	$32,00 \pm 2,31$	b	76,667 \pm	3,528ab	84,667	± 4,667a	$79,333 \pm$	1,764ab	н
	B.b.T-A	$9{,}83 \pm 0{,}97$	e	22 ±	4,163e	52	± 3,055b	51,667 \pm	2,028c	C
	EABb 13/01-Rf	$27,77 \pm 1,08$	bc	83,333 ±	4,807ab	96	± 1,155a	81,333 \pm	0,667ab	
	EABb 13/02-Rf	$41,09 \pm 2,41$	а	88,667 \pm	1,764a	94	± 2a	$88 \pm$	1,155a	
ST "A"	EABb 13/03-Rf	$20,86 \pm 1,43$	cd	$68 \pm$	4,619bc	90,667	± 4,372a	76 ±	6,11ab	
	EABb 13/04-Rf	$47,\!15\pm0,\!60$	а	88,667 \pm	3,333a	94	± 2,309a	$78 \pm$	3,055ab	
	EABb 12/03-Pa	$41,\!45 \pm 1,\!06$	а	87,333 \pm	2,906a	90	± 4,619a	86,667 \pm	4,807ab	
20-30°C	EABb 08/05-Rf	$28,\!19\pm0,\!69$	bc	$56 \pm$	2c	87,333	± 2,404a	75,333 \pm	6,667ab	
	EABb 08/06-Rf	$24{,}79 \pm 3{,}61$	bcd	$78 \pm$	1,155ab	91,333	± 2,404a	85,333 ±	0,667ab	
	B.b.T-N	$27,74 \pm 1,44$	bc	36,667 ±	2,404de	89,333	<u>± 4,807a</u>	82 ±	2ab	



Effect of ultraviolet radiation (UV-B) on conidial germination culturability and colony growth



 B. bassiana strains obtained from RPW in the Mediterranean basin are highly adapted to Mediterranean conditions (environmental competence) explaining high prevalence among RPW (and PBM)





- Molecular study of the isolates:
 - > Elongation factor (EF1- α)
 - > Combined analysis (EF1-α–BLOC)
 - > ISSR
- Effect of humidity, temperature, and UV
- Pathogenicity and virulence against *R. ferrugineus* pupae and adults

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Entomopathogenic fungi for RPW control



Laboratory and semifield trials

B. bassiana isolates have revealed high effectivity against larvae and adult RPW **Cumulative survival ratio** ---Control 0.8 ---- EABb 07/06-Rf ----EABb 12/03-Pa 0.6 ------IBB010 0.4 B.b.T-A ---- EABb 13/04-Rf 0.2 ----EABb 12/04-Rf 0 1 2 3 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 4 5 6 8 7 Days Cumulative Survival Ratio ---CONTROL -07/06-Rf -BbTA 0 12 2 3 5 6 8 9 10 11 1 4 7

Days

iys

Fungi

Beauveria bassiana EABb 07/06-Su strain virulence towards different RPW stages and instars

Probit lines adjusted to mortality data of R. ferrugineus different stages when exposed to B. bassiana in the laboratory.

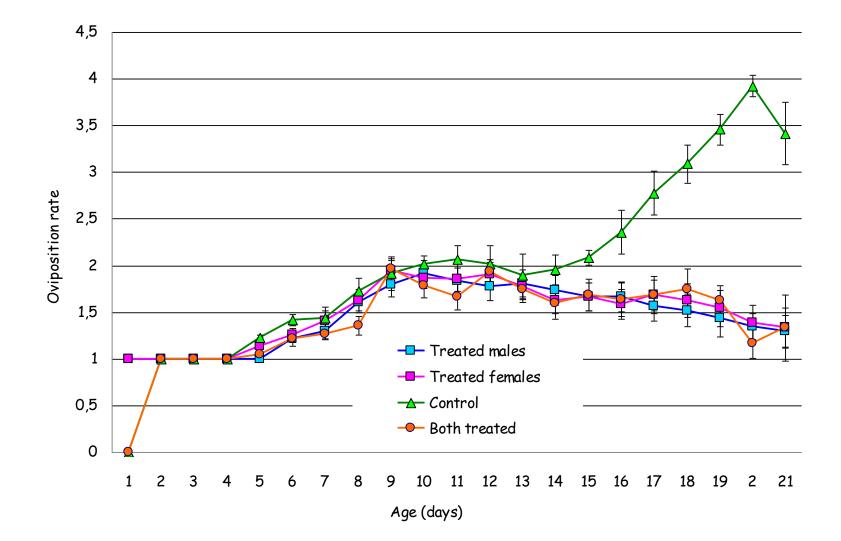
Stage	Time (days)	n	Slope ± SE	χ²	P-value ^a	LC ₅₀ (conidia/ml)	95% FL
Eggs	4	144	0.706 ± 0.194	0.1823	0.9129	1.5×10^{8}	$1.1\times10^75.6\times10^8$
Neonate	4	144	1.384 ± 0.260	0.5184	0.7717	3.7×10^{8}	$1.8 \times 10^8 - 6.5 \times 10^8$
Fourth instar	4	120	1.561 ± 0.246	0.1575	0.9243	6.3×10^{7}	$3.7 \times 10^7 - 1.0 \times 10^8$
Lab adults ^b	21	144	1.471 ± 0.278	0.8021	0.6696	7.2×10^{8}	$3.7 \times 10^8 - 1.2 \times 10^9$
Field adult ^b	21	144	0.861 ± 0.236	0.1217	0.9410	3.0×10^{9}	$1.1 \times 10^9 1.1 \times 10^{10}$



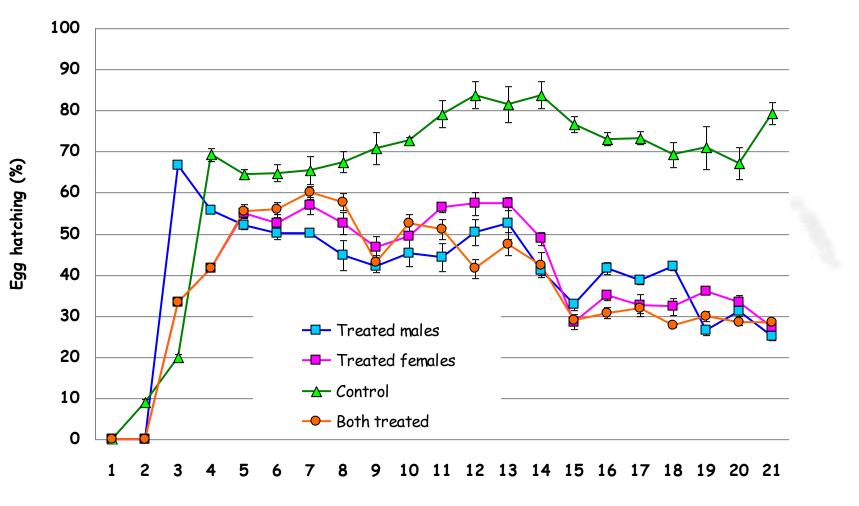
Fungi

But in estimating the full biocontrol potential of Beauveria bassiana it is important not only the direct mortality caused by the treatment but also the sublethal reproductive effects and autodissemination potential highly impacting mid and long term population dynamics

Red palm weevil control by entomopathogenic microorganisms Fungi Lower fecundity of surviving females



Lower egg viability



Age (days)

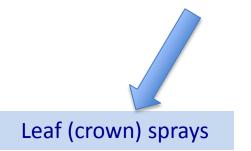
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Entomopathogenic fungi for RPW control

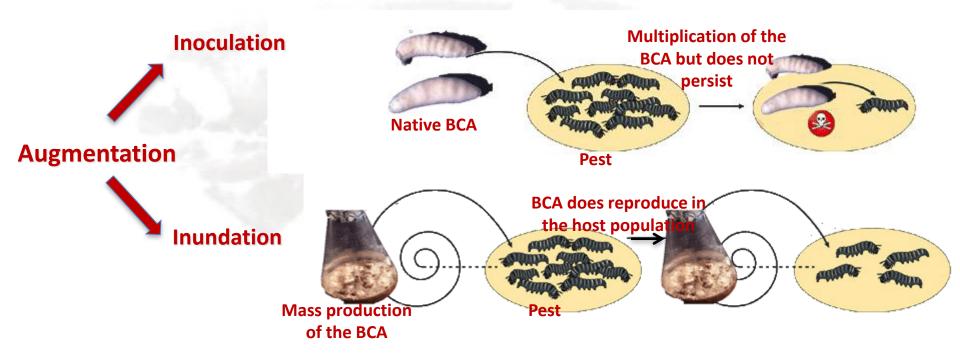


Field evaluation



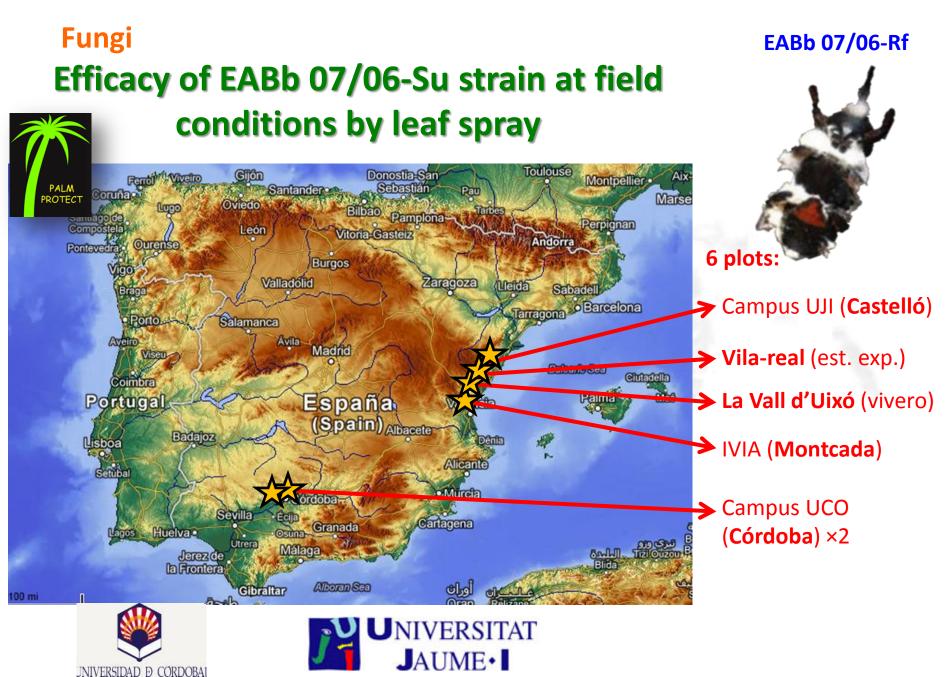
Strategies for the use of microbial control agents for pest control

iiMycoinsecticidesiii



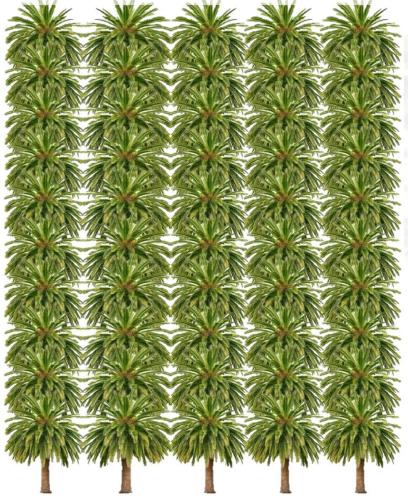
BCA: Biological Control Agent

Modifies from: Roderick y Navajas. 2003. Genes in new environments: genetics and evolution in biological control. Nature Reviews Genetics 4, 889-899.



Fungi

Efficacy of EABb 07/06-Su strain at field conditions by leaf spray









Fungi

Efficacy of EABb 07/06-Su strain at field conditions



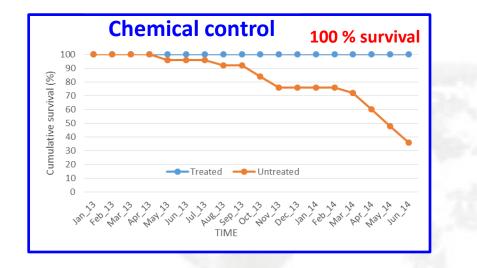
Chemical control (monthly)

 Beauveria bassiana (EABb 07/06-Rf)

- Monthly
- Bimonthly
- Three-monthly

Jan 2013-Jun 2014

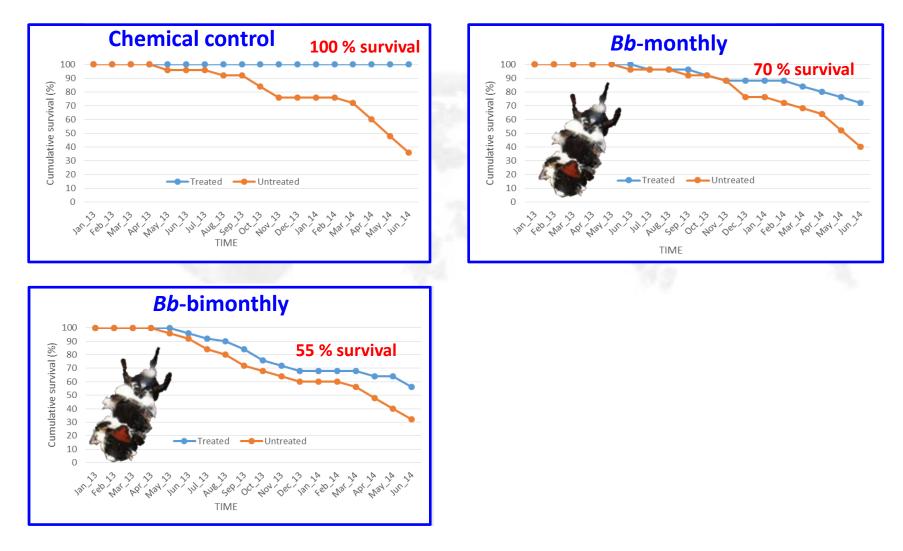
Fungi



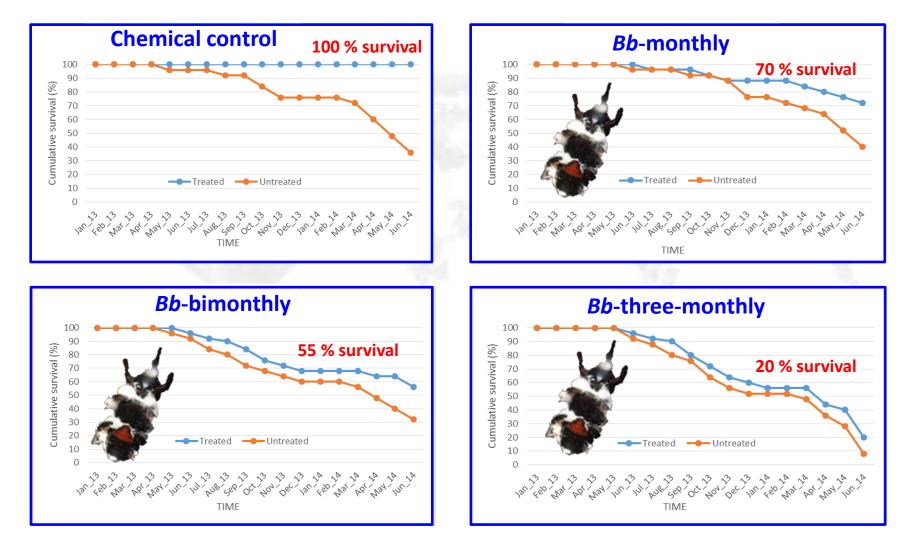
Fungi



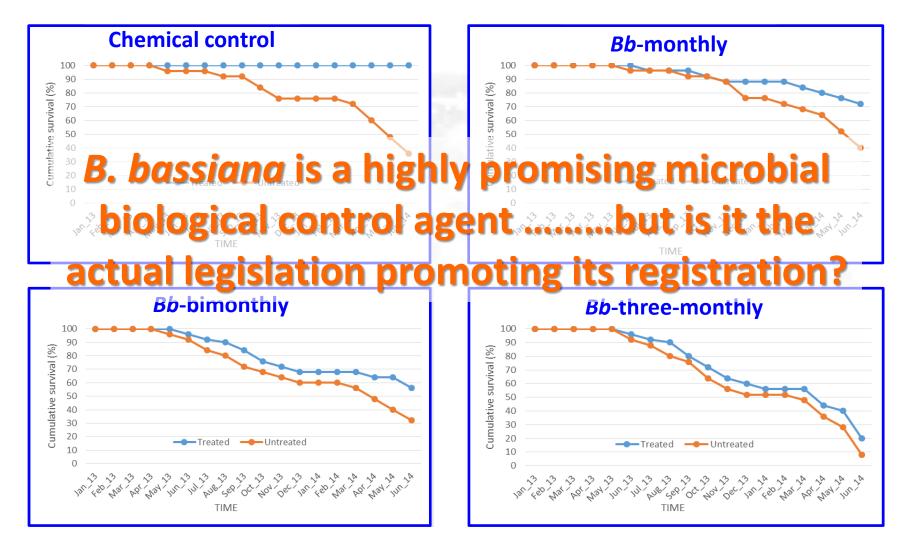
Fungi



Fungi



Fungi



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Entomopathogenic fungi for RPW control



Lure and kill

Efficacy of EABb 07/06-Su strain at field conditions by the lure & kill (attract-and-infect) strategy



EABb 07/06-Rf

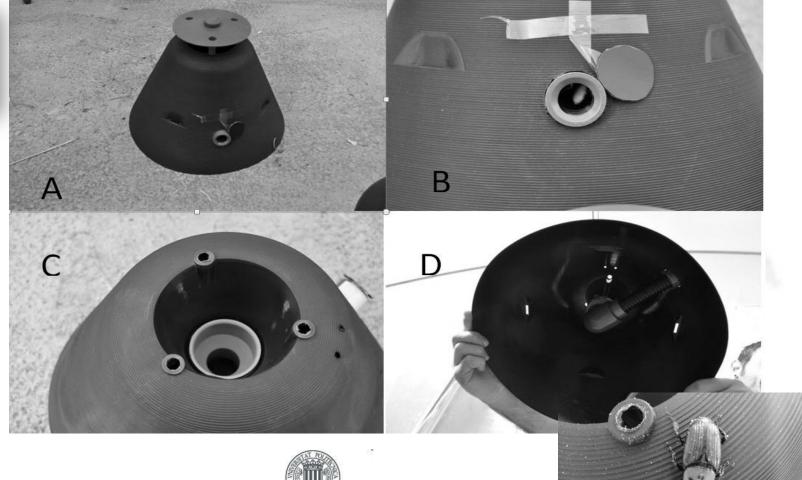


Since R. ferrugineus adults are mobile they can vector the fungus to less reachable areas and spread it to cryptic individuals and/or life stages, which are otherwise hard to eradicate.



Design of a better trap based on existing "Picusan" trap to be developed as a attract and infect device (lure & kill device)

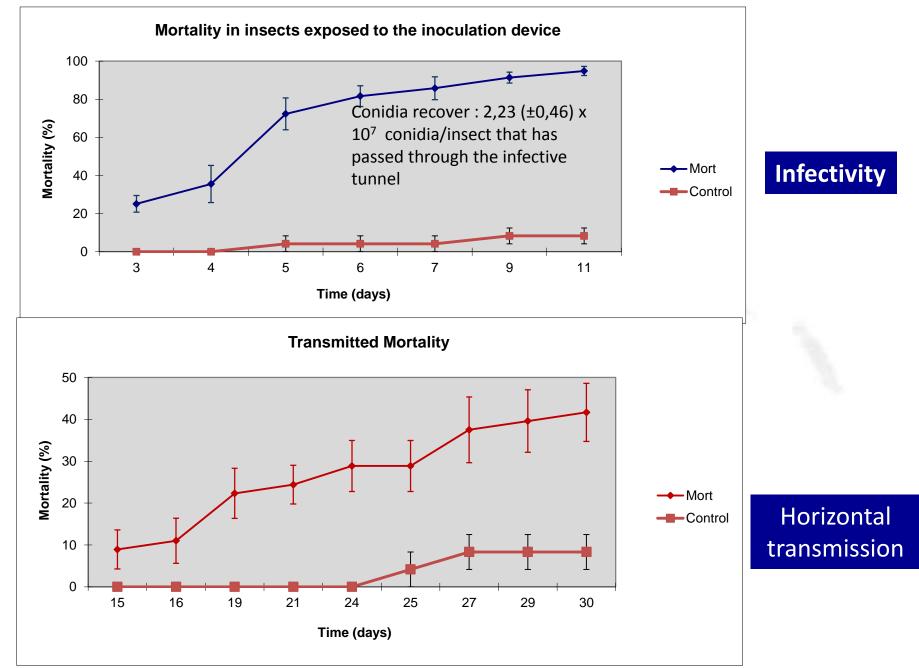








Ongoing work.....promissing.....



Handmade lure & kill device



Arundo donax L. (poales:poaceae)





Arundo donax Cane inoculated with EABb 07/06 Rf











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Entomopathogenic fungi for RPW control



Insecticidal compounds from EPF

EMA are able to produce high-molecular-weight proteins or low molecular weight secondary metabolites that can also applied against *R. ferrugineus* adults and





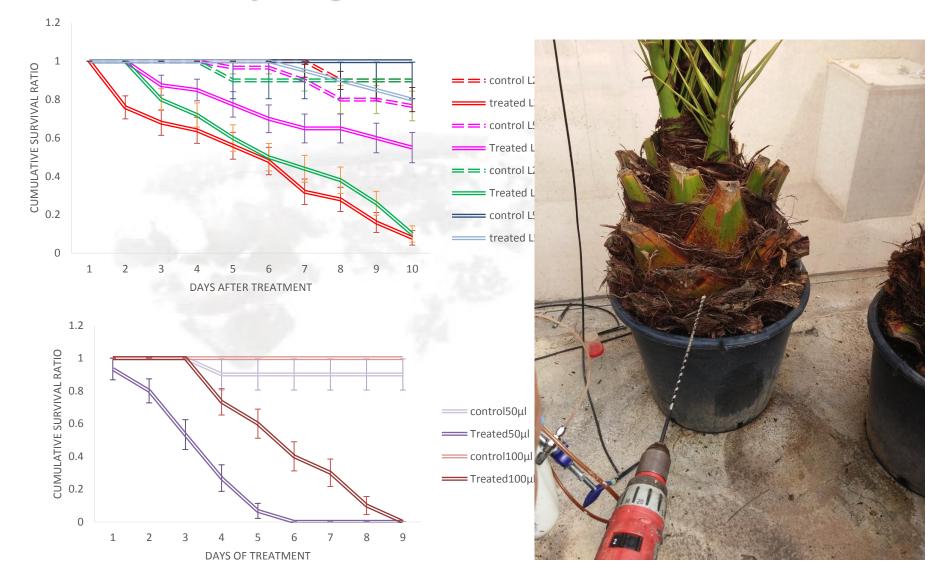








Evaluation of EAMb 09/01-Su strain extract against *R. ferrugineus* larvae and adults



Acknowledgments

✓ To my team members Lola Ortega, Natalia González, Carlos
 Campos e Inmaculada Garrido

✓ To Prof. Jaques-Miret and Dr. Oscar Dembilio

✓ To Prof. Jaime Primo and Dr. Vicente Navarro

✓To the the institutions funding the research, especially the Valencian Department of Agriculture (CAPA), the Spanish Ministry of Science and Innovation (MINECO) and the EU Commission.

✓ To FAO for this opportunity



Many thanks for your attention.....