Metarhizium and *Beauveria* as endophytes: what species are really colonisers and is there potential for deployment in commercial systems

Travis Glare

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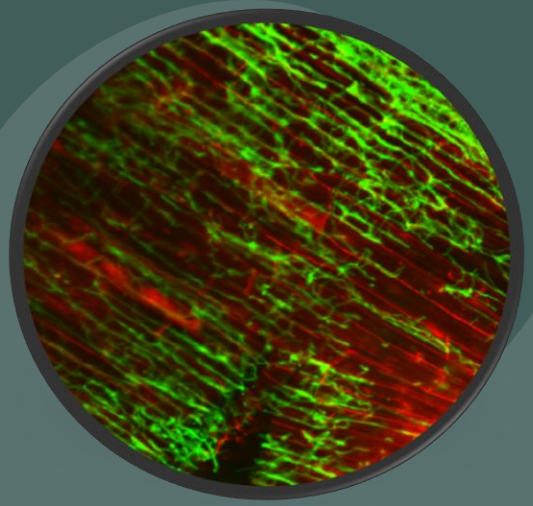
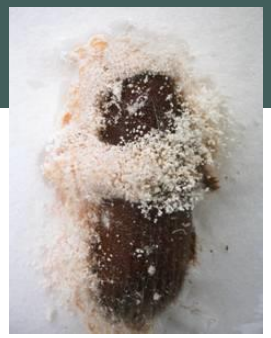
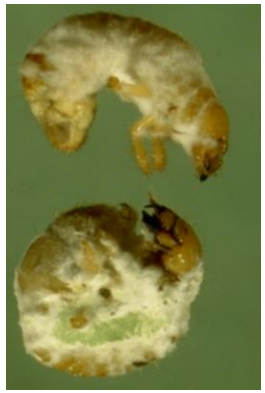


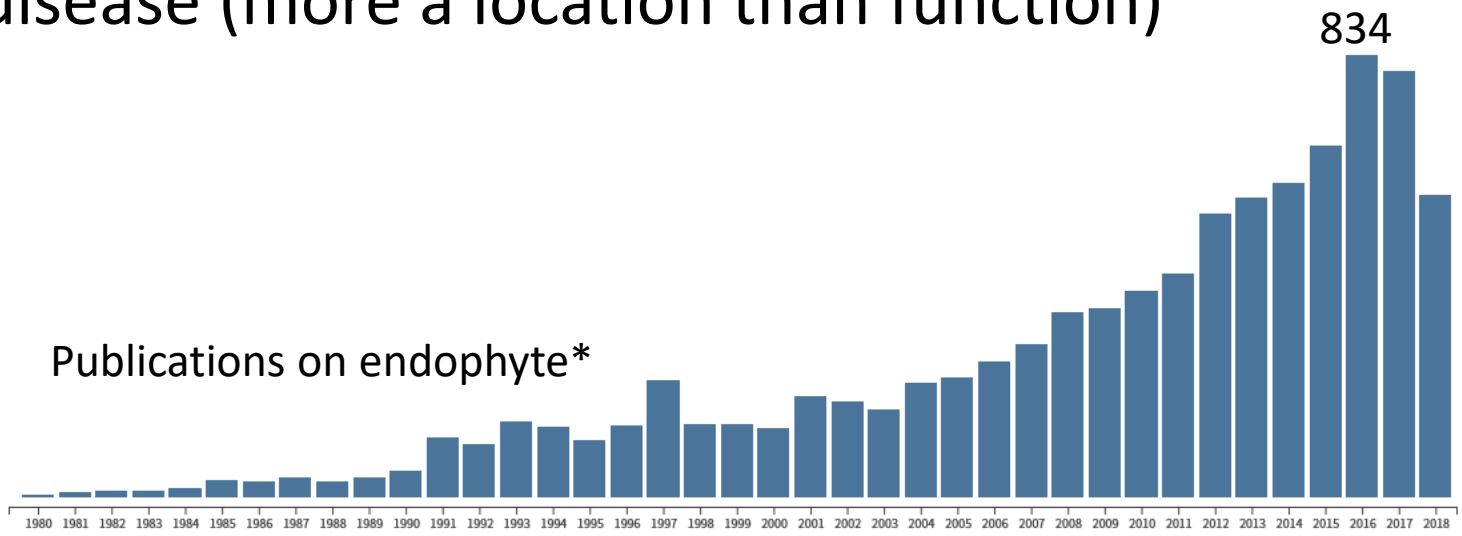
Beneficial microbes





Pathogen- kills invertebrates, plants or other microbes: *Beauveria* and *Metarhizium* well known insect pathogens

Endophyte –living within a plant without causing disease (more a location than function)



Endophytic beneficials- artificially introduced

Lecanicillium lecanii *Aspergillus parasiticus*	Cotton, wheat, corn, bean, tomato, and pumpkin
Hypocrea lixii *Gibberella moniliformis* *Fusarium oxysporum* *Trichoderma asperellum*	Bean
Clonostachys rosea *Trichoderma harzianum* *Trichoderma atroviride* *T. asperellum* *H. lixii*	Onion
Purpureocillium lilacinum	cotton
Metarhizium robertsii	Sweet sorghum
Isaria fumosorosea	Tomato
Metarhizium anisopliae	Broad bean and cassava
Metarhizium pingshaense	Corn
Metarhizium brunneum	Broad bean, potato, sweet pepper

And *Beauveria bassiana…*

Reviewed in Jaber and Ownley **Biological Control** 116, 2018, 36-45

Beauveria bassiana establishment as endophyte

Banana *Musa* spp.
Bean *Vicia faba*
Cocoa *Theobroma cacao*
Corn *Zea mays*
Jute *Corchorus olitorius*
Pine *Pinus* spp.
Cotton *Gossypium* sp.
Strawberry *Fragaria X ananassa*
Tomato *Solanum lycopersicum*
Date Palm *Phoenix dactylifera*
Cassava *Manihot esculenta*
Artichoke *Cynara scolymus*
Cotton *Gossypium* sp.
Wheat *Triticum aestivum*
Pumpkin *Cucurbita maxima*
Grapevine *Plasmopara viticola*
Squash *Cucurbita pepo*
Rice *Oryza* sp.
Opium poppy *Papaver somniferum*
Sorghum *Sorghum bicolor*

Onion *Allium cepa*
Coffee *Coffea Arabica*
Cauliflower *Brassica oleracea* var. *botrytis*
Tobacco *Nicotiana tabacum*
Soybeans *Glycine max*
Oil seed rape *Brassica napus*
Sweet pepper *Capsicum annum*
Jimsonweed *Datura stramonium*
Potato *Solanum tuberosum*
Cocklebur *Xanthium strumarium*

B. brongniartii as endophyte of beans *Vicia faba*
Jaber & Enkerli (2017) Biocontrol Science and Technology, 27:1, 28-41

Metarhizium plant associations

Metarhizium more commonly found associated of plant roots, rather than endophytic.

Metarhizium robertsii, *Metarhizium brunneum* and *Metarhizium guizhouense* associate with plant roots

- *M. robertsii* only one associated with grass roots
- *M. guizhouense* more prominent on sugar maple
- *M. brunneum* with more common shrubs and trees

Endophism has been reported:

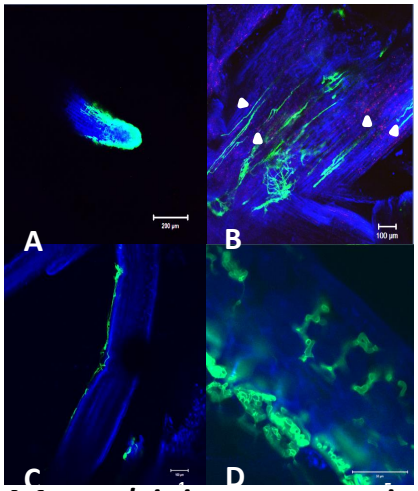
Metarhizium spp. was confirmed in oilseed rape (Batta, 2013), potato (Ríos-Moreno et al., 2016), broad bean (Jaber and Enkerli, 2017), alfalfa, tomato and melon (Resquín-Romero et al., 2016, García et al., 2011), corn (Kabaluk and Ericsson, 2007

Wyrebek et al. 2011 Microbiology (2011), 157, 2904–2911

Mode of action

Endophytic entomopathogens can negatively affect insect herbivores but mechanisms behind these effects remain largely unknown, but could involve:

- Plant defence response
- Direct infection (rarely report)
- Bioactive metabolites of *B. bassiana* and *Metarhizium* (e.g. destruxin)



Metarhizium on maize

Limited evidence of vertically-transmitted fungal endophytes (Quesada-Moraga et al., 2014, Lefort et al., 2016)

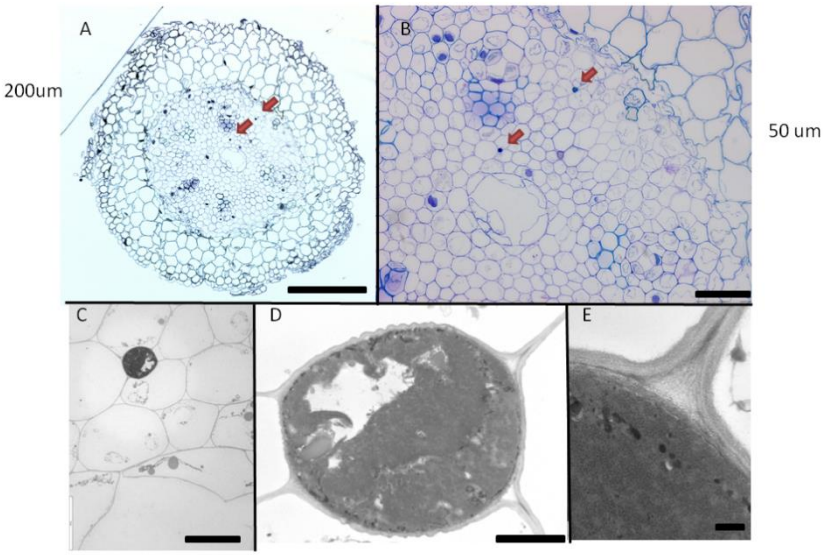
Photo by Federico Rivas 2018

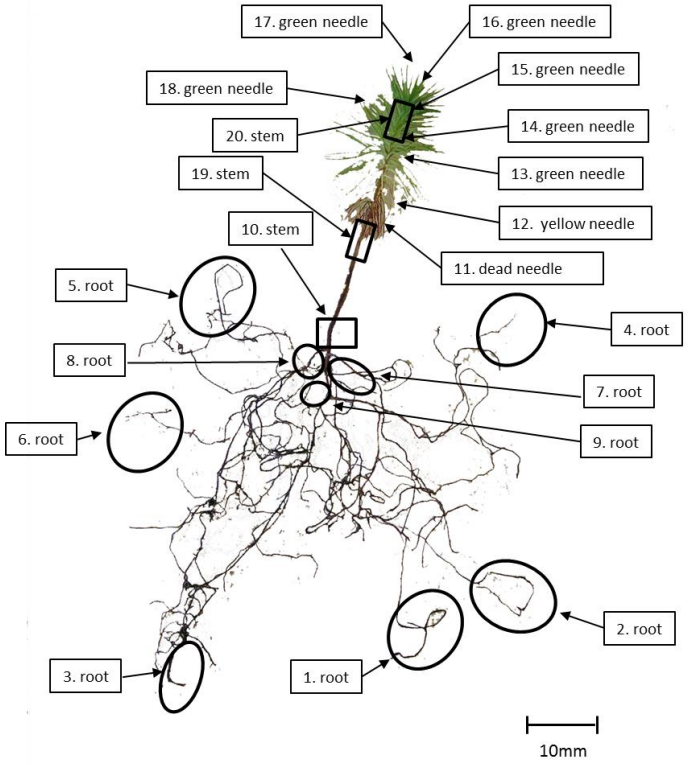
Beauveria in pines

Went throughout the seedlings

Very low amount of hyphae?

Can be vertically transmitted





Brookes, McKinnon and Glare

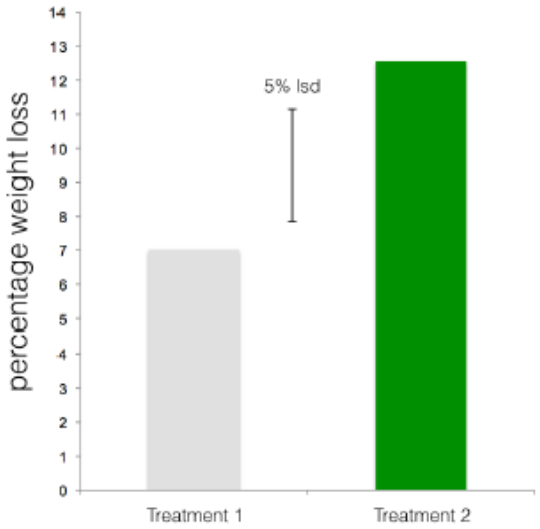
Is it infective or toxic to insects when in plants?

Using 2 of 30 pines from an infected seed batch, feeding trials conducted

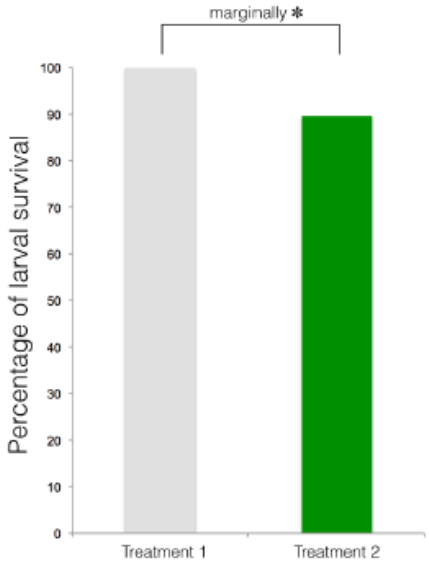
Grass grub larvae- feed root pieces

Results: caused weight loss in grass grub

Average larval weight loss



Larval survival



MC LeFort, T Glare, J Brookes and T Nelson 2016 NZ plant protection

Not always an effect

Beauveria bassiana as an endophyte in *Arabidopsis* was not antagonistic to *Plutella xylostella* and *Myzus persicae*.

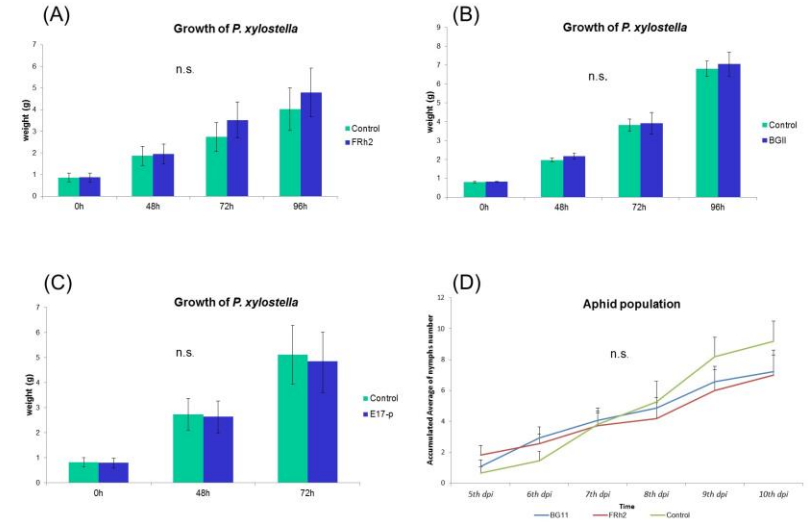
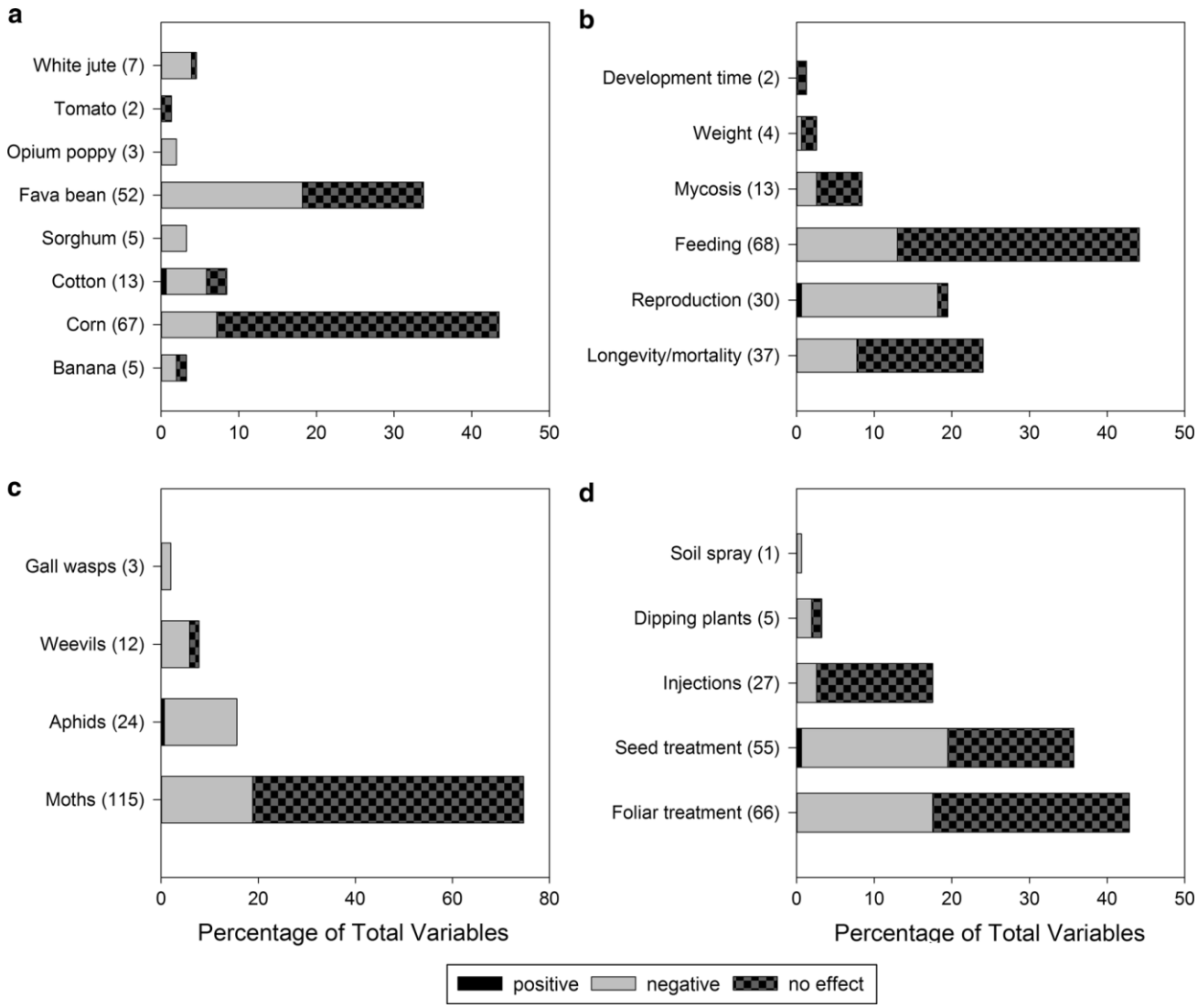


Figure 2: Effect of endophytic *B. bassiana* on herbivores: (A-C) Endophytic *B. bassiana* effects on *P. xylostella* growth post-infestation with third instar caterpillars. Error bars represent the standard error of the mean (N=15-20). Caterpillars of the same age were used within one experiment. (D) Endophytic *B. bassiana* effects on *M. persicae* population after 10 days post infestation. Error bars represent the standard error of the mean (N=12-14). No significant differences between treatments ($P < 0.05$). n.s. = not significant.

Data from Maya Raad, SIP 2015

Beauveria bassiana



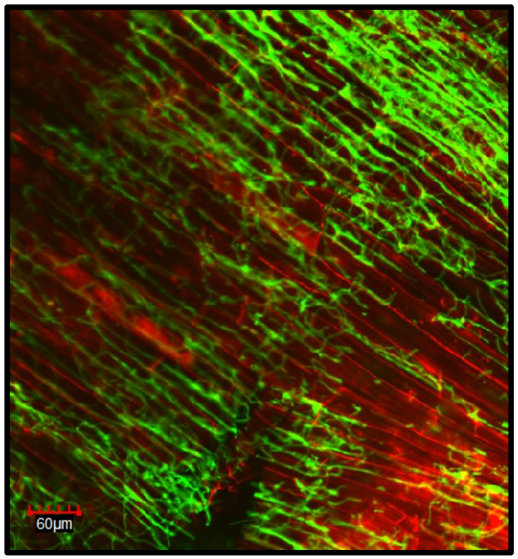
Effects of endophytic *Beauveria bassiana* on herbivores, calculated as percentage of the total number of experimental variables measured (154), from 17 independent articles. Number of variables evaluating each component is in parentheses on the Y axes. Plots show the proportion of total observations across plant species (a), the effect measured (b), the insect herbivore studied (c) and the inoculation method used (d)

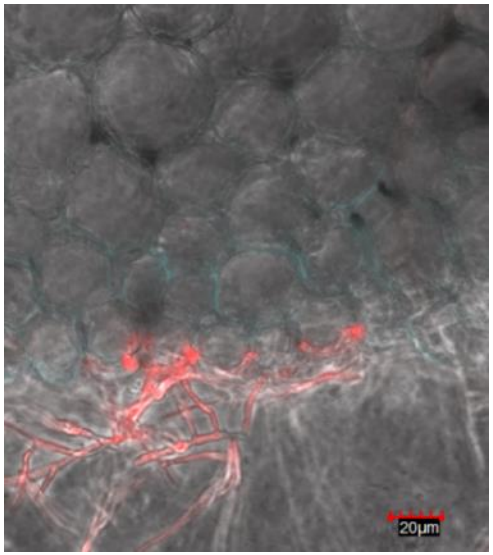
McKinnon et al. 2017 *Beauveria bassiana* as an endophyte: a critical review on associated methodology and biocontrol potential. BioControl (2017) 62:1–1

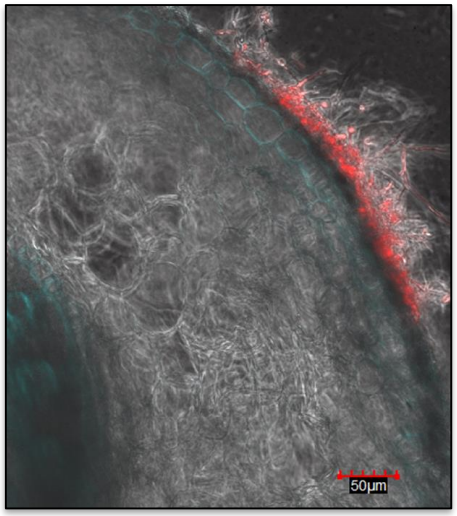
Not always truly endophytic

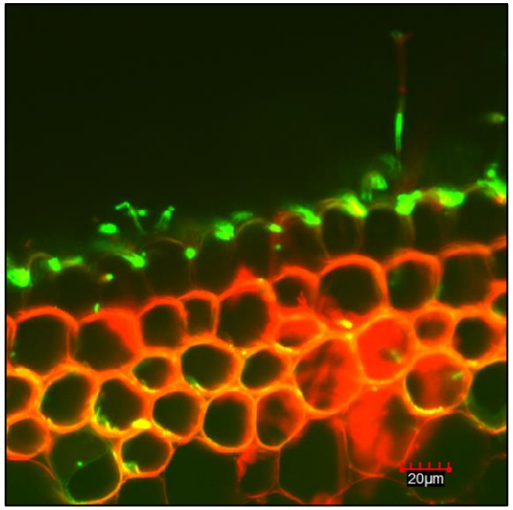
Epiphytic
Endophytic
Rhizospheric

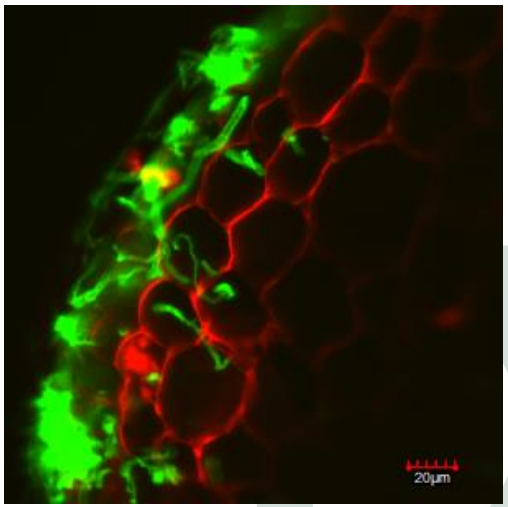
Beauveria in maize







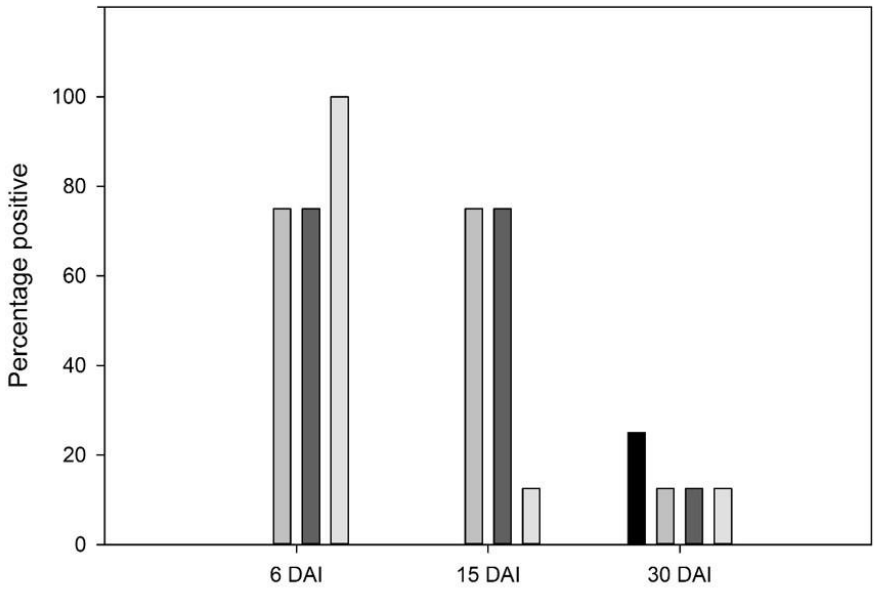




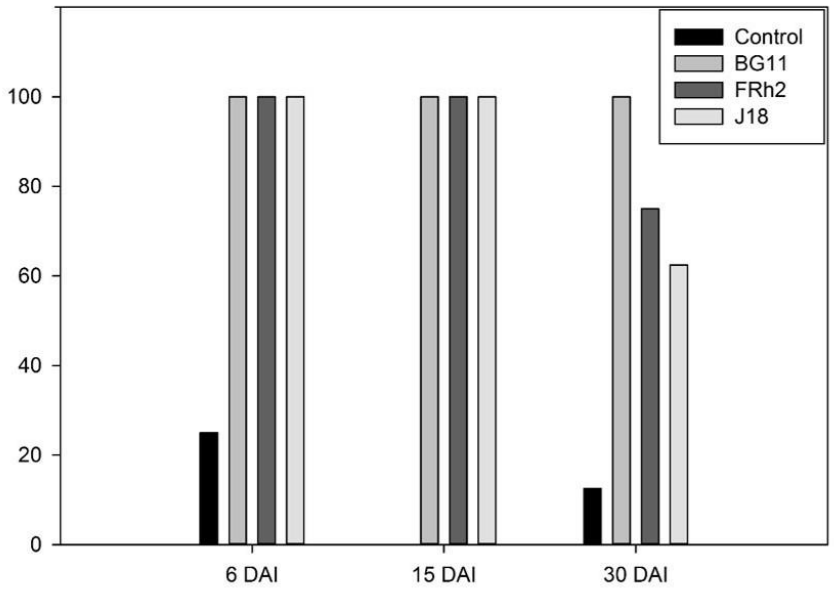
Moran-Diez at al., in prep

Beauveria bassiana in the rhizosphere

Detection in soil



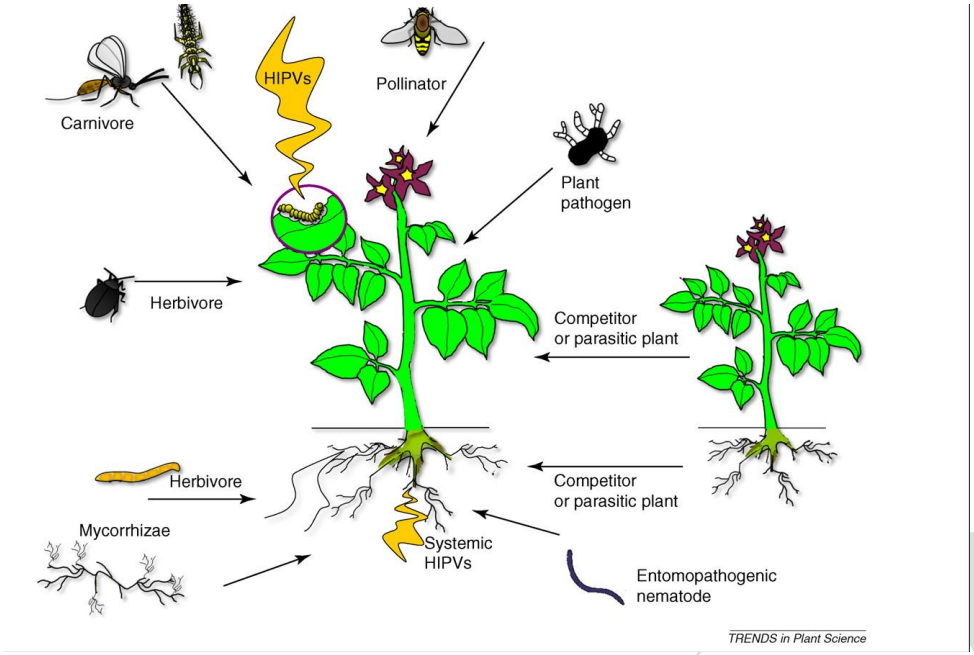
Detection in/on roots



- PCR *ef1α* detection

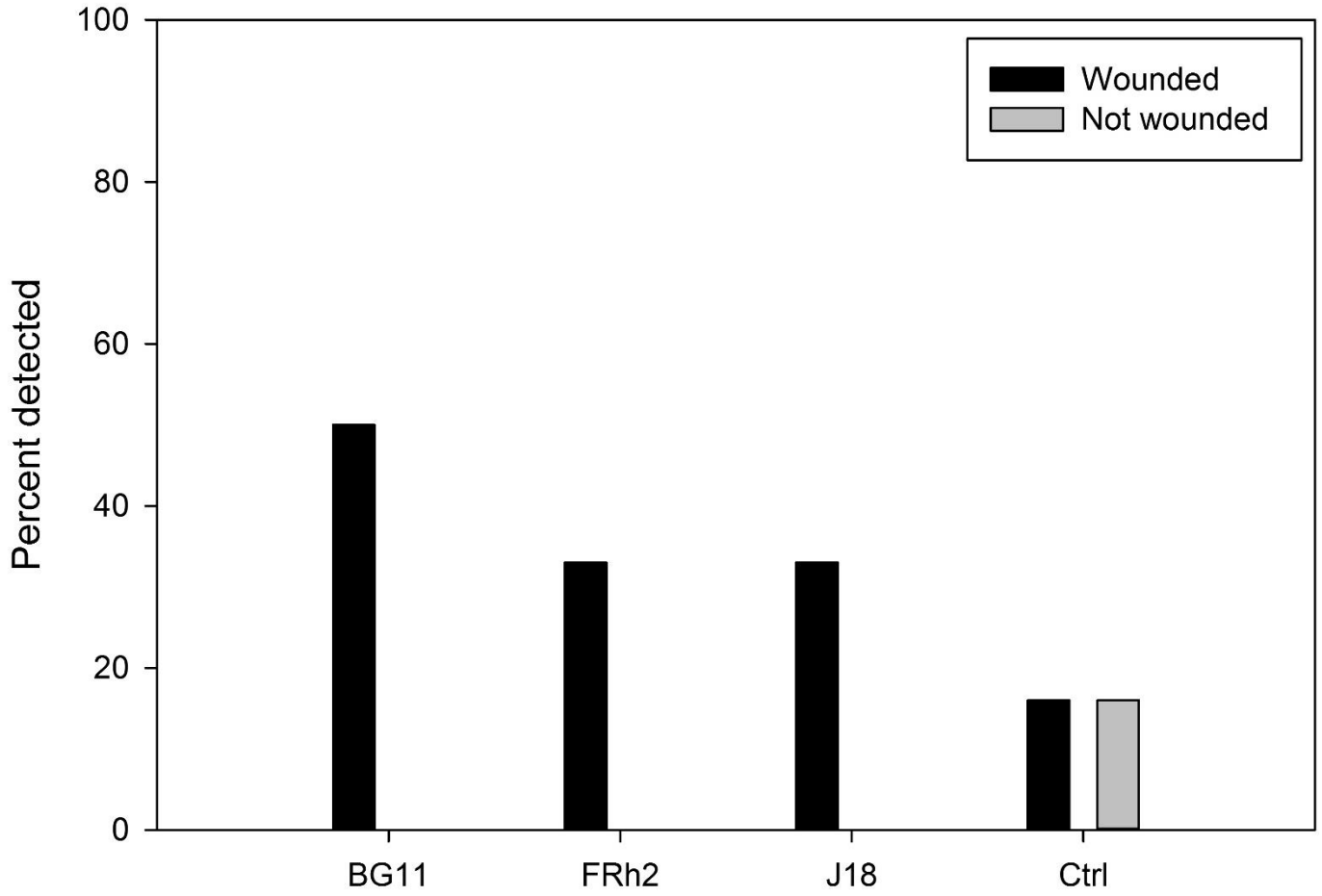
Plants are not static in biocontrol

- Plant attraction and architecture
- Plant response and defence
- Plant volatiles and root exudates
- Microbes as bodyguards

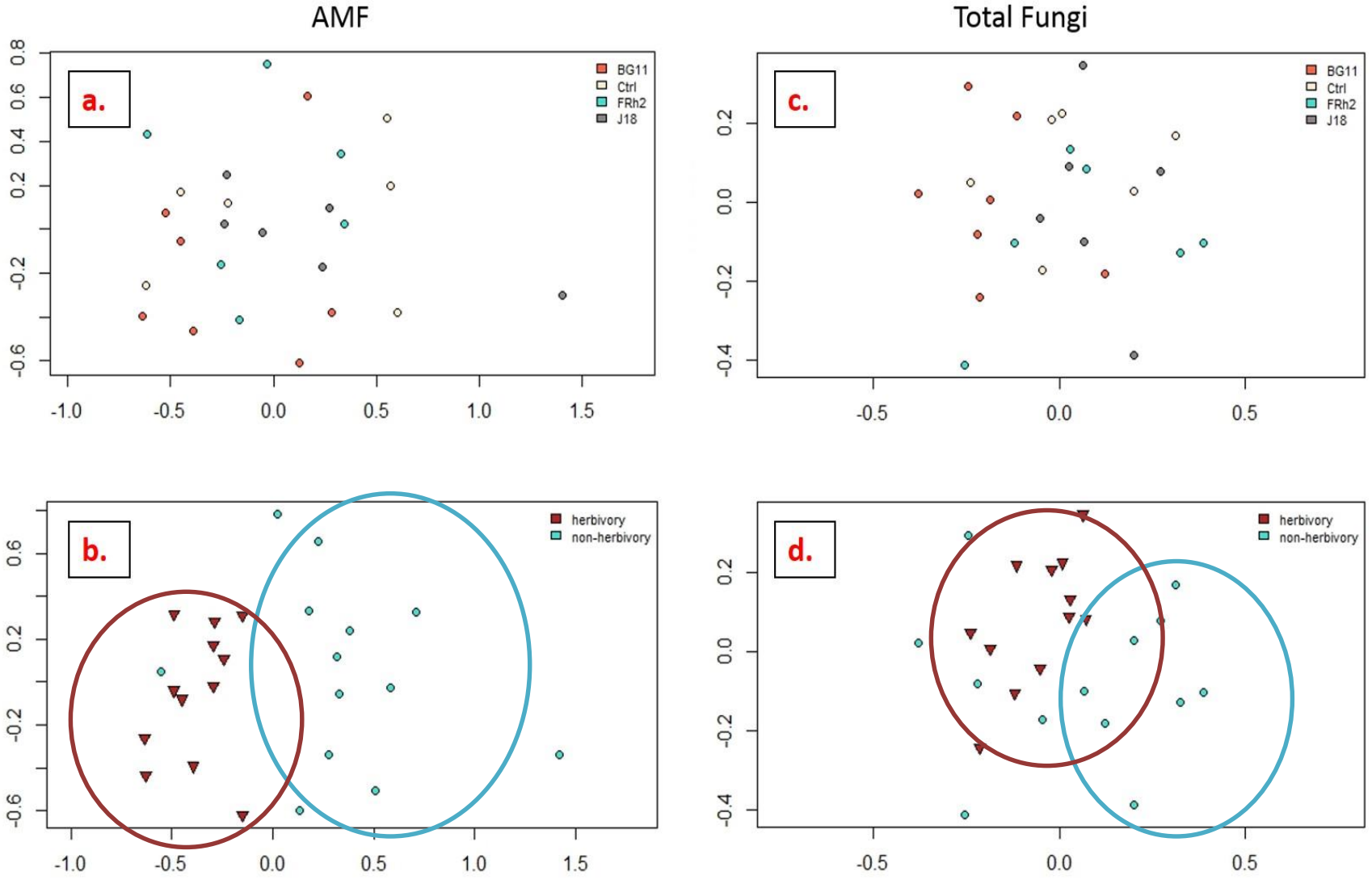


Dicke and Baldwin 2010 Trends in Plant Science

Retention of *Beauveria* in the rhizosphere after 30 days enhanced by simulated insect herbivory



A significant positive difference on *Beauveria* detection frequency found as a result of the simulated herbivory treatment (wounding of foliage) once all reps were processed (September 2017) (**P = 0.019)** (McKinnon *et al.* in review)



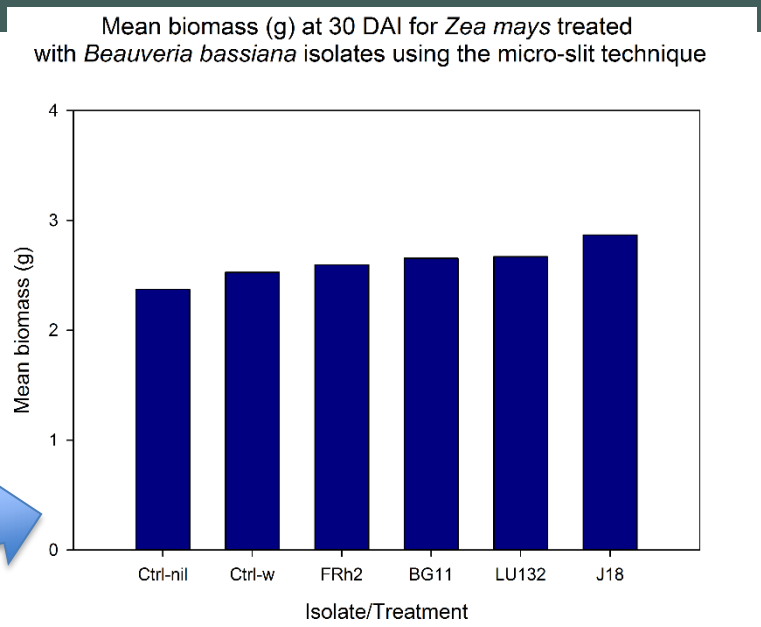
Target group	Linear R^2	Non-metric R^2	Stress
Arbuscular Mycorrhiza Fungi (AMF)	0.68	0.96	0.20
Fungi	0.80	0.94	0.24
Alphaproteobacteria	0.77	0.95	0.21
Betaproteobacteria	0.72	0.95	0.22

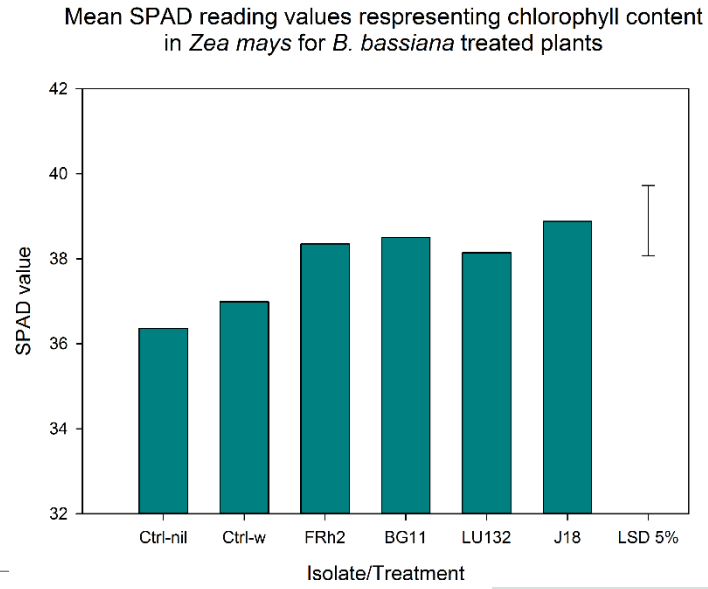
Impact on plants – *B. bassiana* & *Zea mays*

Used *B. bassiana* isolates colonising maize after wound inoculation.

B. bassiana presence can affect the growth of the plant, both positively or negatively.

Chlorophyll content was increased in most cases.





Aimee McKinnon

Metarhizium as plant growth promoter

M. brunneum, M. anisopliae, and *M. robertsii* significantly increased corn:

- leaf collar formation (by 15, 14, and 13 %),
- stalk length (by 16, 10, and 10 %),
- average ear biomass (by 61, 56, and 36
- average stalk and foliage biomass (by 46, 36, and 33 %)

- Produce plant-growth-promoting auxins on roots
- Possible antimicrobial effects
- Root colonization necessary for benefits

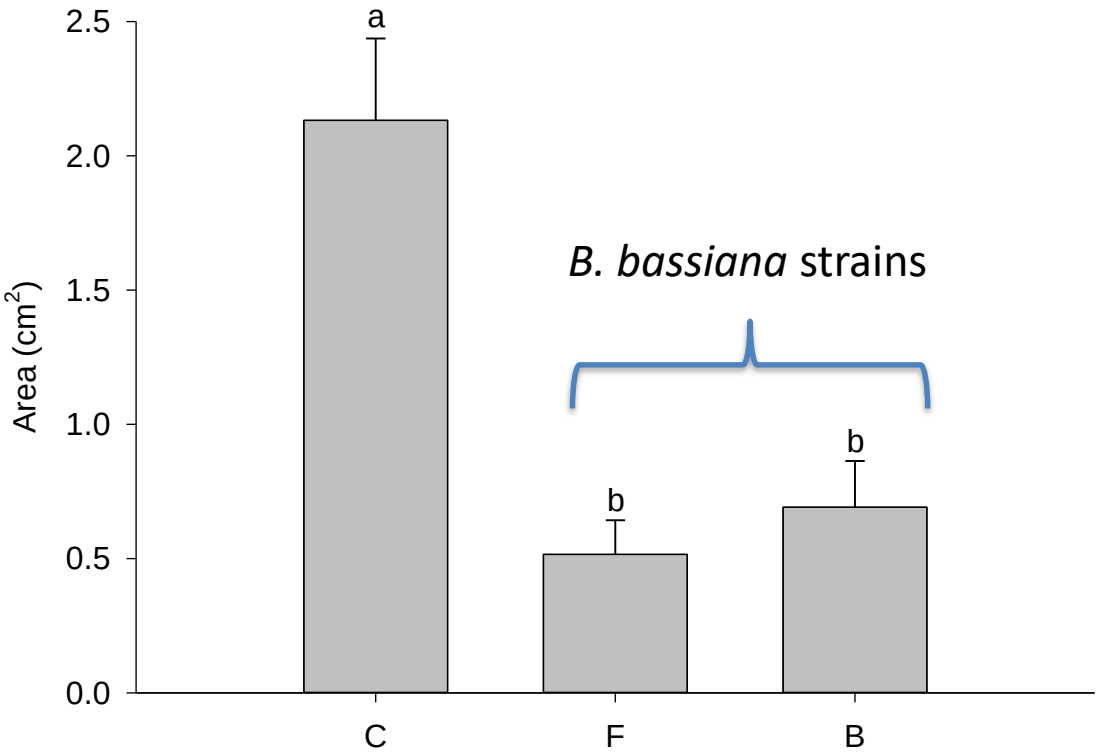
Liao et al. Appl Microbiol Biotechnol (2014) 98: 7089

Against plant pathogens

There is now substantial evidence that some endophytic fungal entomopathogens, particularly *B. bassiana* and *Lecanicillium* spp. demonstrate antagonistic activity against plant pathogens

Jaber and Ownley **Biological Control** 116, 2018, 36-45

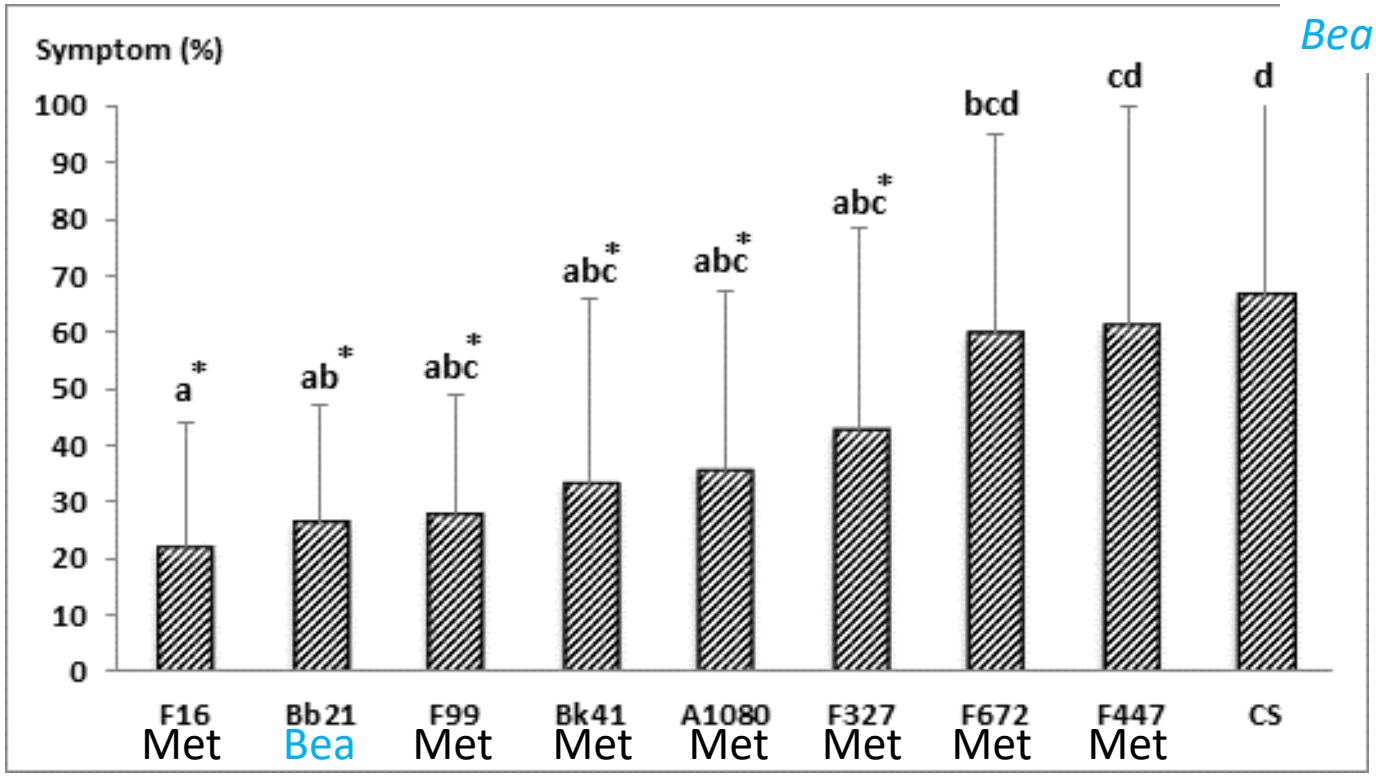
B. bassiana as an endophyte reduces plant disease



Leaf area infected with *Sclerotinia sclerotiorum* measured in *Beauveria bassiana* colonised (F = FRh2 and B = BG11) and control (C) *Arabidopsis thaliana* plants 5 days post infection. Disease intensity was calculated as average lesion area.

Reduction in *Fusarium* infection

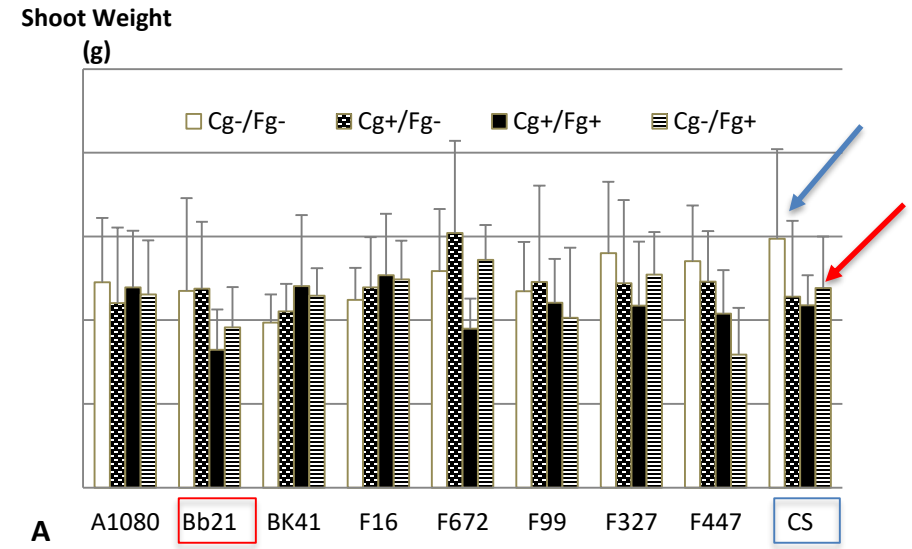
Met = *Metarhizium*
Bea = *B. bassiana*



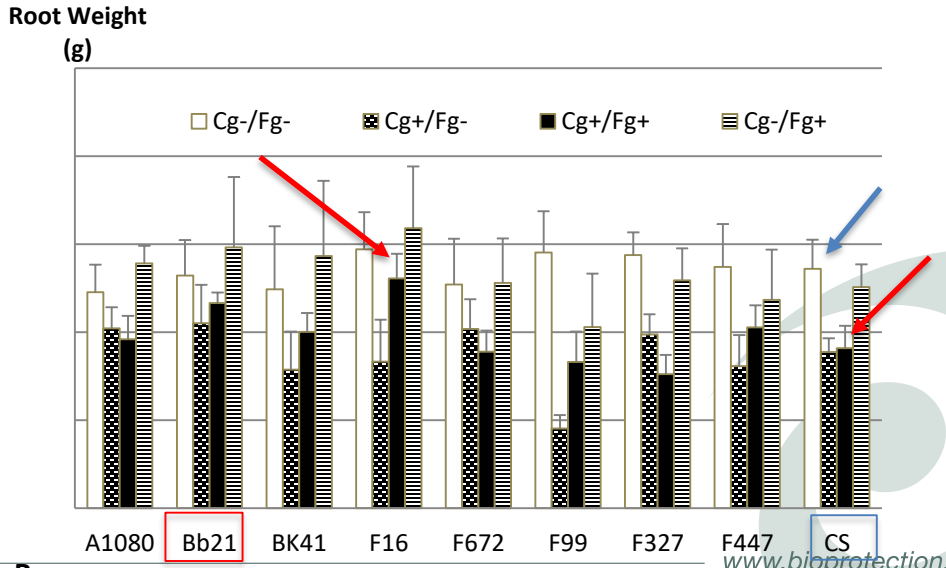
Maize root symptom of *Fusarium graminearum* infection for plants grown from seeds coated with entomopathogenic fungi.

Metarhizium and *Beauveria* applied to maize

Plant maize dry weight after seed coating with different fungi grown in the presence of *Costelytra giveni* (Cg) and *Fusarium graminearum* (Fg).



A



B

Rivas et al. in prep.

Transcriptomic analysis of maize and *Arabodopsis* in response to root colonization by *B. bassiana*

- J18
  (from *Zea mays*)

- FRh2
  (from a pine bark beetle cadaver)

- BG11
  (from dandelion roots)

RNA obtained at 3 DAI

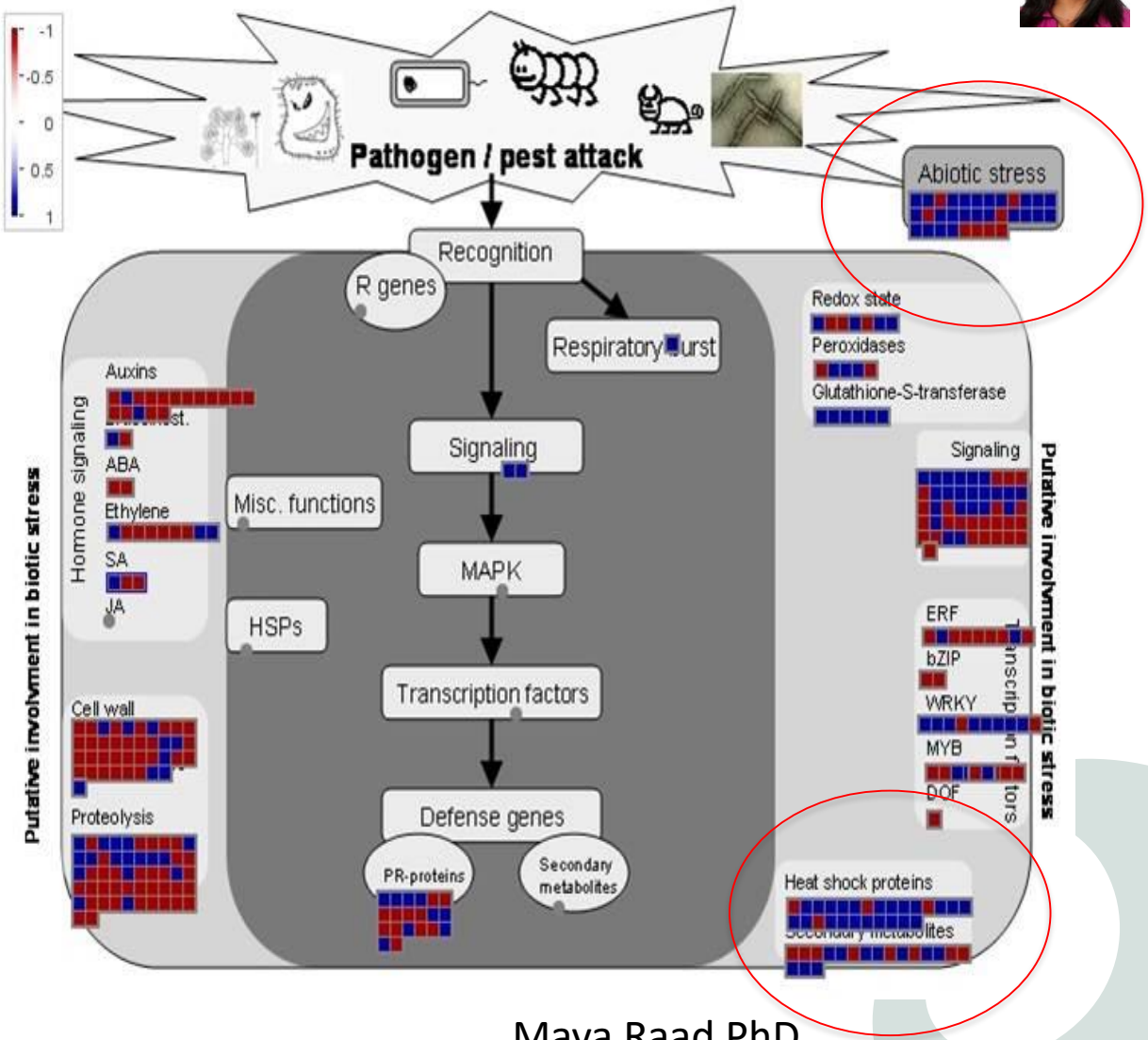
Differentially expressed genes (DEGs)

Probe Set ID[1]	Gene Title[2]	Indicated Function[3]	Control	BG11	J18	Log2 Fold Change (BG11 vs. J18)	P value
Zm.298.1.S1_a_at	Dxr protein	Plant defence - terpenoid biosynthesis	754	510	658	-1.11	0.0424
ZmAffx.12.1.S1_at	Kaurene synthase 2	Plant defence - terpene biosynthesis	619	268	593	-3.44	0.00078
Zm.8714.1.A1_at	Acc oxidase (ethylene-forming enzyme)	Plant defence - signalling	305	212	369	-2.39	0.00388
Zm.10830.1.S1_at	BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase 1	Plant defence - signalling	56	37	57	-1.79	0.01181
Zm.948.1.A1_at	Receptor-like protein kinase	Plant defence - signalling	176	148	203	-1.36	0.01443
Zm.7462.1.A1_at	NAC domain-containing protein 21/22	Plant defence - signalling	96	72	108	-1.75	0.0186
Zm.18148.1.A1_at	Protein kinase	Plant defence - signalling	43	43	54	-0.96	0.04193
Zm.5036.1.A1_at	Serine/threonine-protein kinase NAK	Plant defence - signalling	289	234	291	-0.95	0.04358
Zm.6659.1.A1_at	Pathogenesis related protein-5	Plant defence - SAR salicylic pathway	736	515	1461	-4.51	0.00206
Zm.15280.1.A1_s_at	Pathogenesis related protein4	Plant defence - SAR salicylic pathway	1588	594	1160	-2.9	0.01716
Zm.411.1.A1_at	Nucleoredoxin1	Plant defence - regulation response to oxidative stress	704	598	827	-1.4	0.01361
Zm.18344.1.A1_at	Major facilitator superfamily defense1	Plant defence - metabolite transport	111	77	233	-4.79	0.00138
Zm.499.1.S1_at	Hypersensitive induced reaction3	Plant defence - cell death/lesion response	728	672	937	-1.44	0.01866
Zm.11896.1.A1_at	SNF1-related protein kinase regulatory subunit beta-1	Plant defence - ATP-binding	17	15	20	-1.2	0.02051
Zm.1663.1.A1_at	VQ motif family protein	Plant defence - regulation response to oxidative stress	180	144	214	-1.71	0.00703
Zm.16973.1.S1_at	VQ motif family protein	Plant defence - regulation response to oxidative stress	118	92	124	-1.3	0.01636
Zm.5565.1.S1_at	Cysteine protease1	Plant defence - protein degradation	4795	5019	6377	-1.04	0.03032

Arabidopsis response to *B. bassiana* colonisation

B. bassiana endophytically colonizes *A. thaliana.*

- Not antagonistic to caterpillar or aphid
- Levels of jasmonic and salicylic acid did not vary.
- Transcriptomic response included upregulation of stress related genes and other defense pathways.

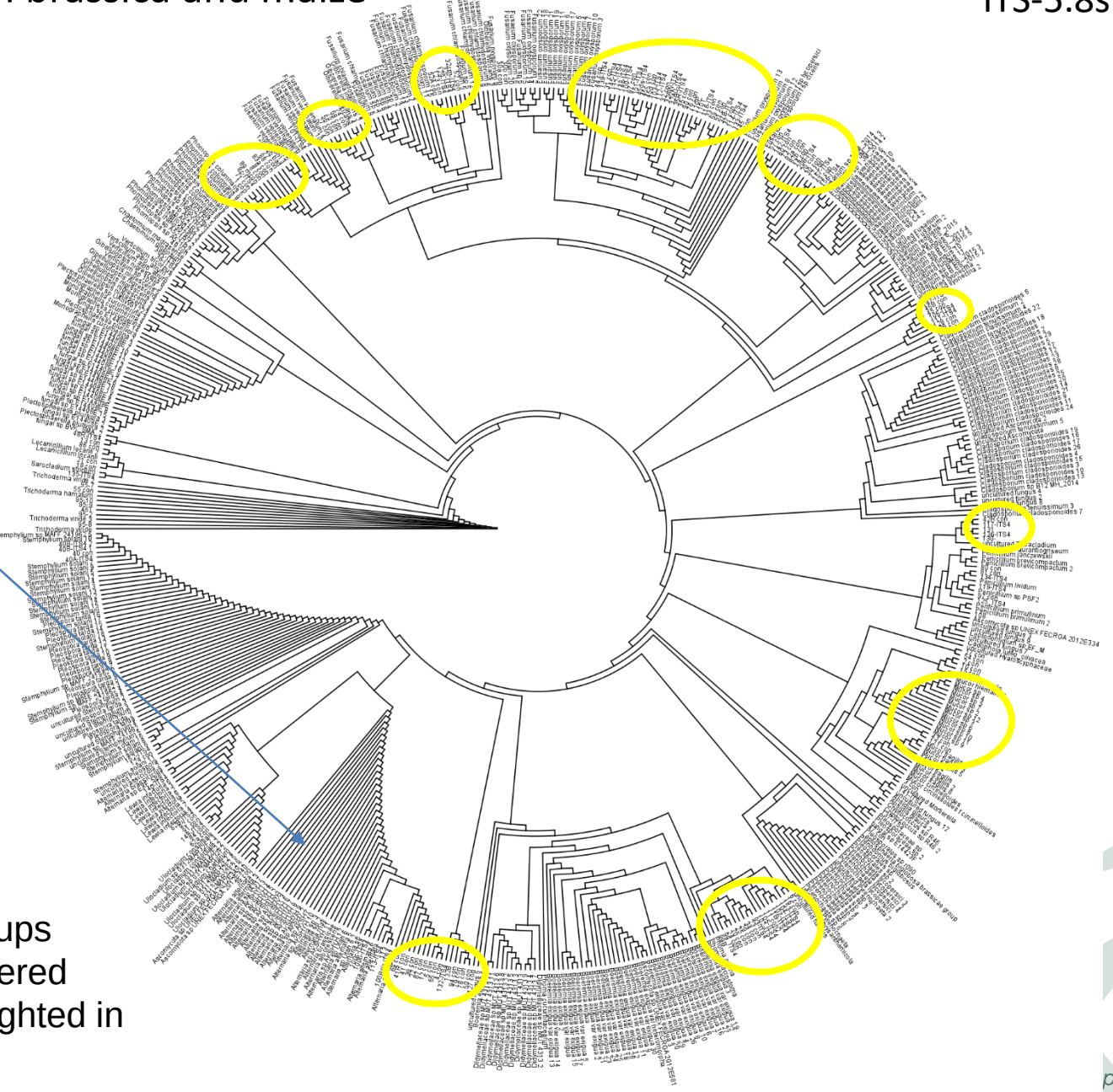


Maya Raad PhD

Endophytes in brassica and maize

ITS-5.8s sequences

Alternaria group



Some isolate groups specifically recovered from maize highlighted in yellow

Potential of endophytes

Some evidence of beneficial effects from some plant-isolate combinations

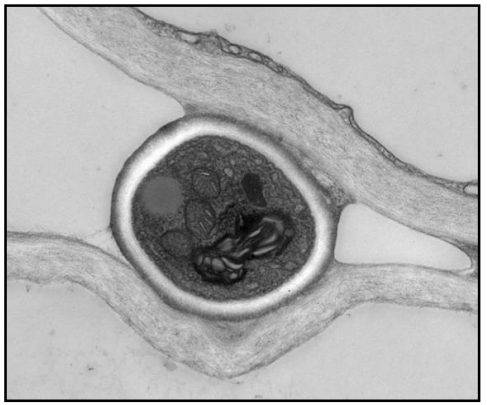
Not as strong as the *Epichloe*-grass interaction

Lack of extensive colonisation? (could this be modified?)

Mode of action largely unknown

But multiple benefits, plant growth stimulation, insect and disease reduction

Part of an IPM system



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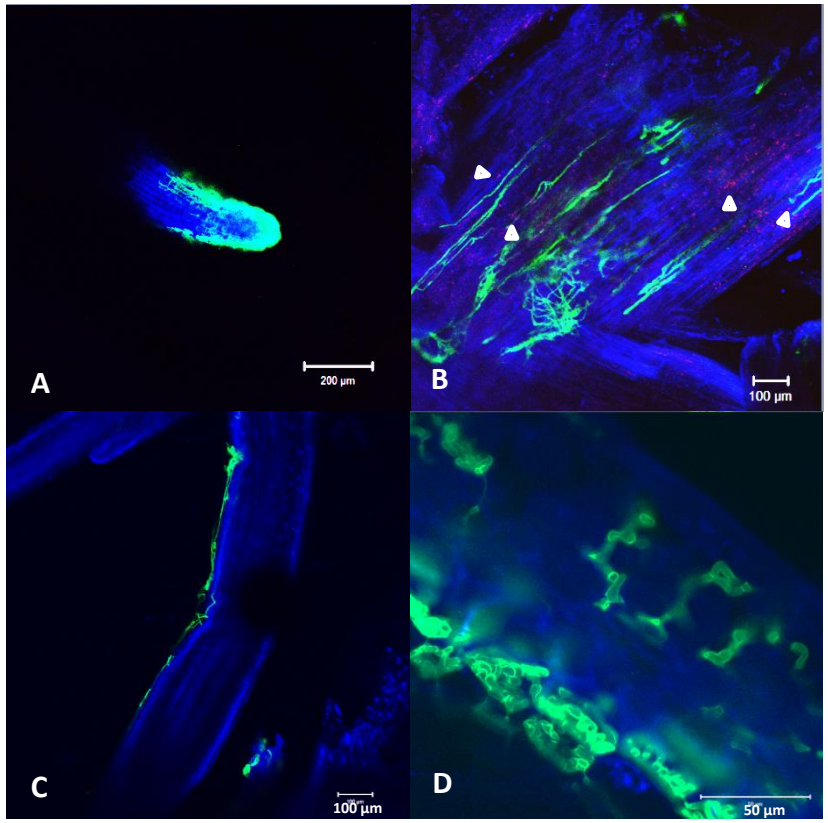

Metarhizium anisopliae-GFP marker associated with the rhizosphere in two weeks old maize plants.

Stained with ConA-AF633 (arrows) to identify hyphal penetration and adhesion sites (red) and propidium iodide to visualize vegetal cell walls (blue).

Photo by Federico Rivas 2018