FH Bielefeld
University of
Applied Sciences

INBIOSOIL
Innovative biological products for soil pest control

Innovative strategies in biological control

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Background

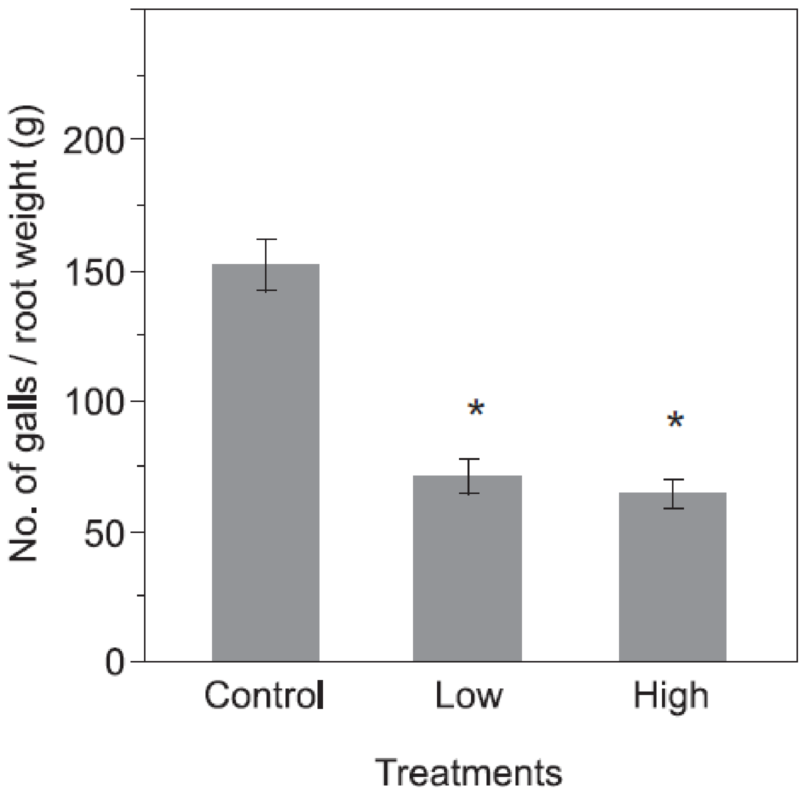
- Political background for implementation of biological control options better than ever
- Public awareness important
- New techniques/methodologies available
- CGR for BCAs at 15 % annually

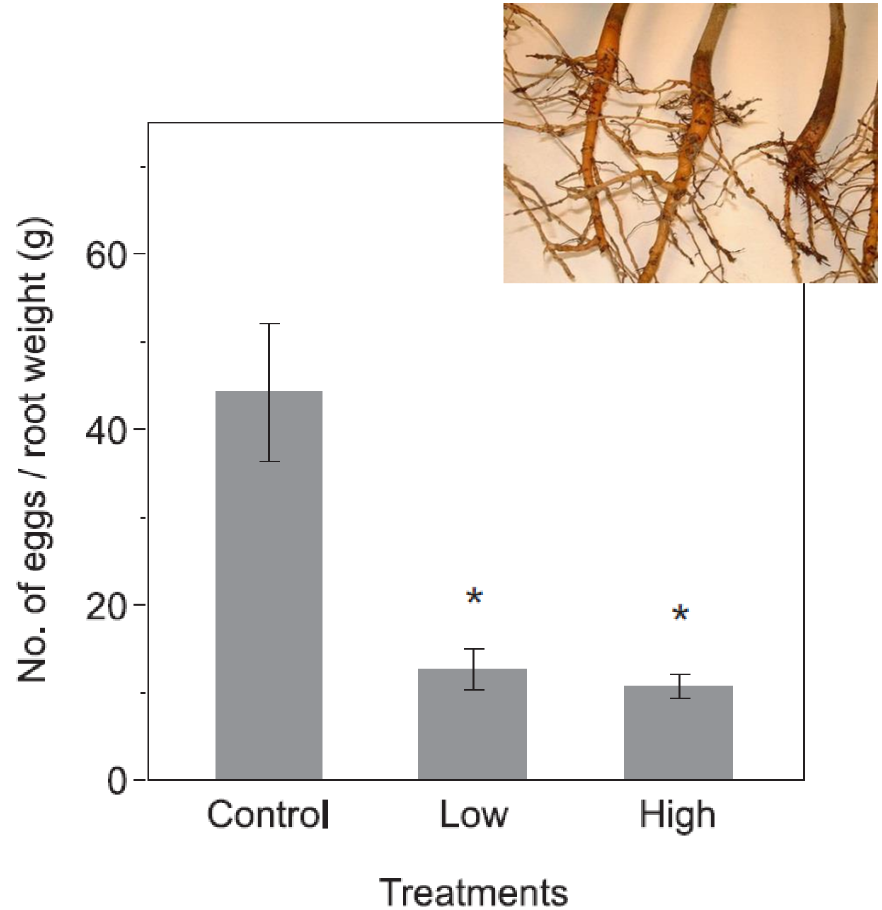
Endophytic fungi as biocontrol agents

What do we know about endophytic fungi?

- Do not cause visible symptoms when colonizing plants
- Found in virtually all plant species (and algae, and ….)
- Producers of an extraordinary array of metabolites
- Plant growth promoters; antagonists of plant pathogens (and ….)

The fungal endophyte *Chaetomium globosum* negatively affects root knot nematodes on cotton

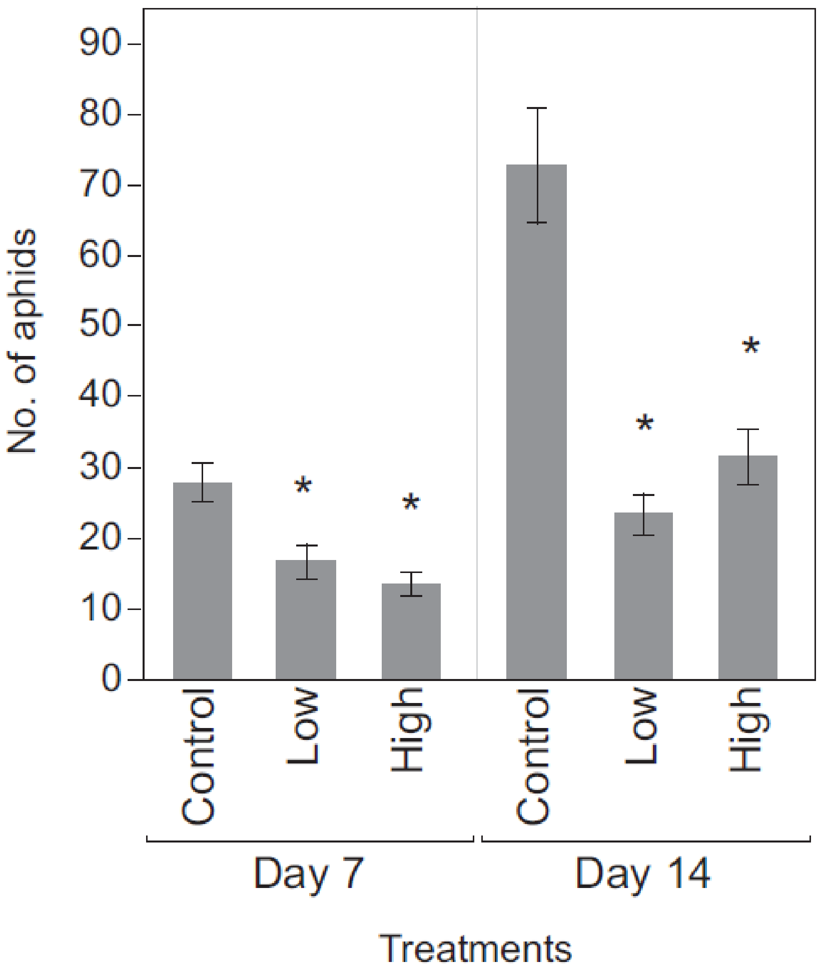


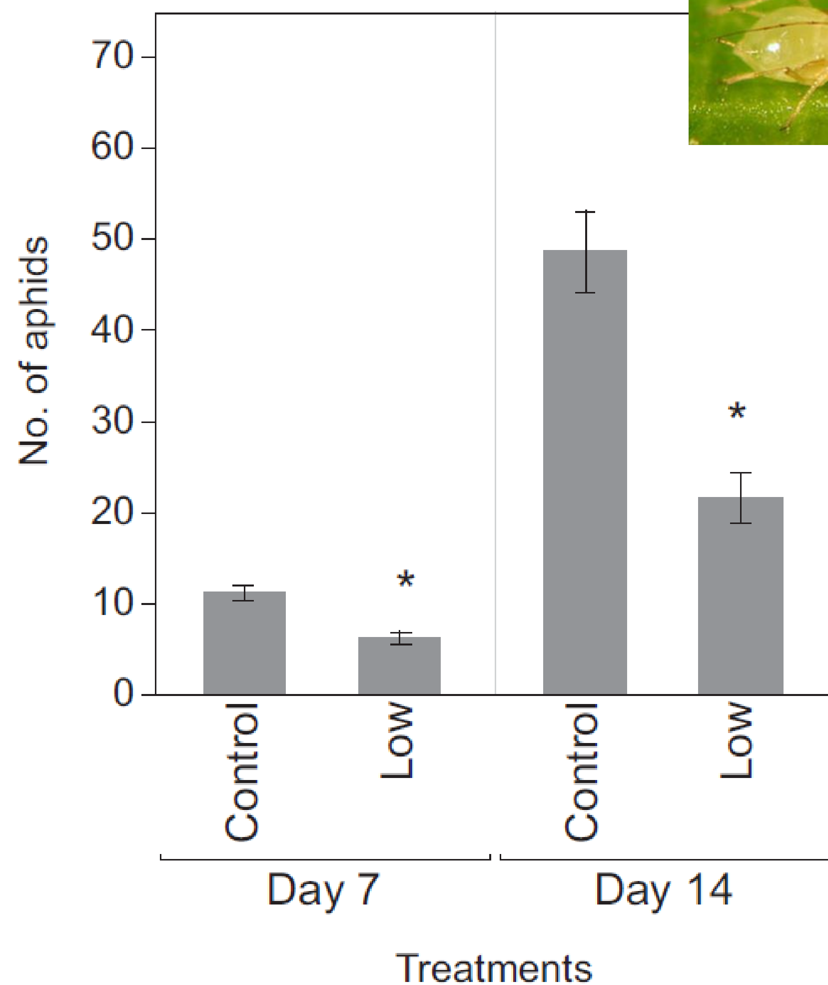


Low = 10^6; High = 10^7

Zhou et al. 2016 FEMS Microbiol Ecol

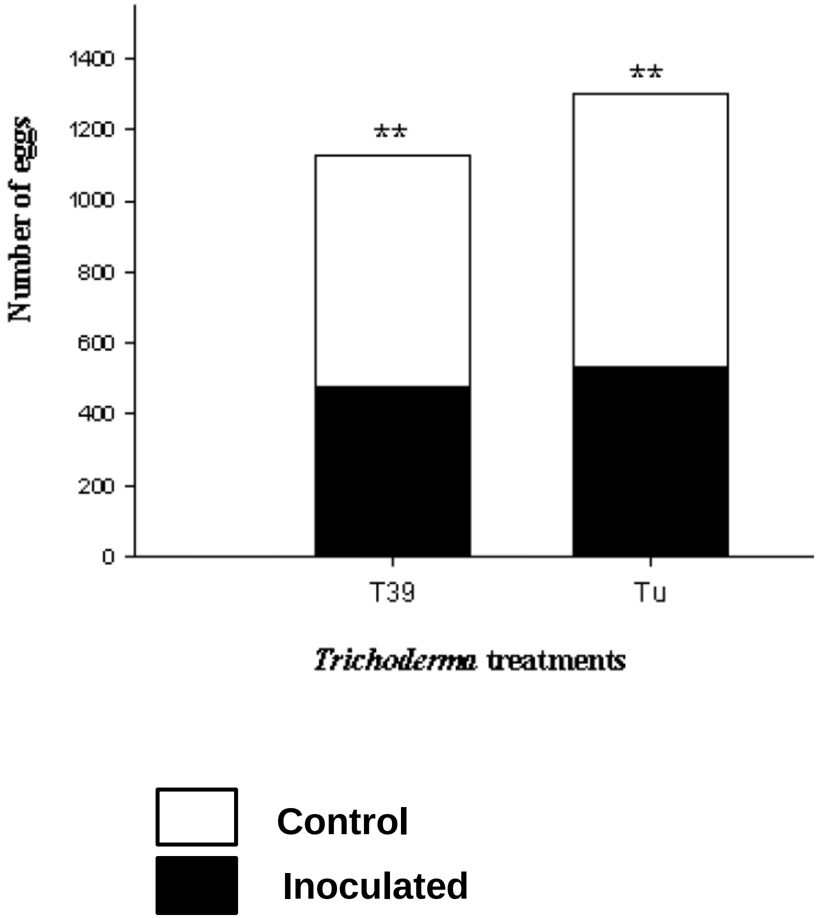
C. globosum also negatively affects cotton aphid reproduction

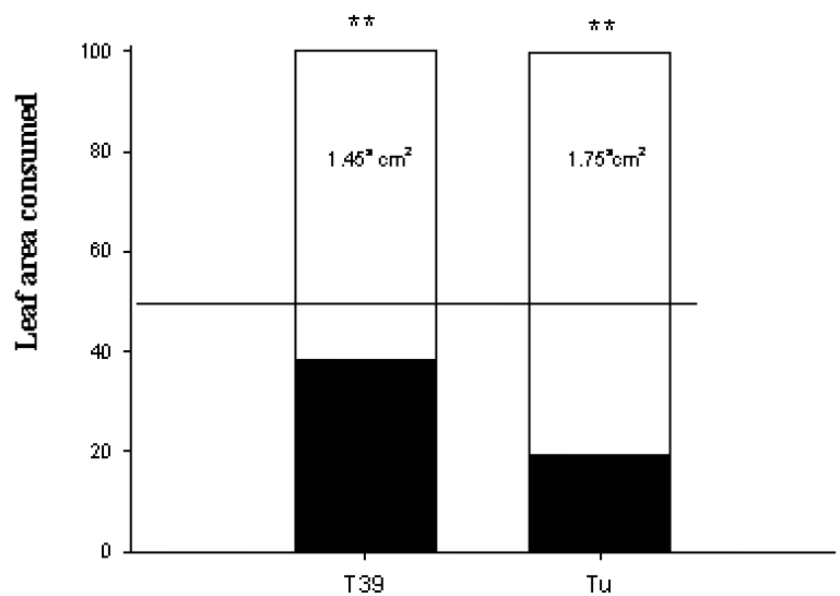




Zhou et al. 2016 FEMS Microbiol Ecol

Oviposition and feeding of Diamondback moth larva on *Trichoderma spp.* inoculated cabbage plants in dual-choice assays





Suryanarayanan et al. 2016 Curr Bioechnol

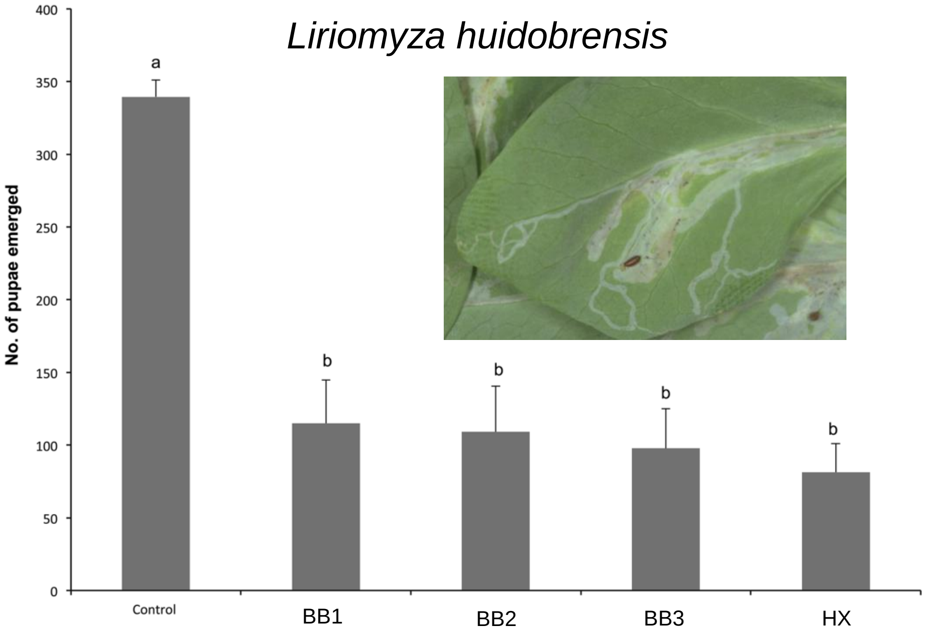
Virulence of 12 *B. bassiana* endophytic isolates/strains against third instar *H. armigera* larvae fed on leaves of inoculated *V. faba* plants

Treatment *B. bassiana* isolate	Parameter sampled ± SE		
	Mortality (%)	Mycosis (%)	Survival time (days)
ATP01	70.00 ± 0.11 ab[a]	00.00 ± 0.00 b	10.36 ± 0.82 b
ATP02	85.00 ± 0.08 a	100.00 ± 0.00 a	6.41 ± 0.58 a
ATP03	10.00 ± 0.07 cd	00.00 ± 0.00 b	23.5 ± 1.50 cd
ATP04	30.00 ± 0.11 bcd	16.76 ± 0.17 b	21.33 ± 0.67 cd
ATP05	40.00 ± 0.11 abcd	37.50 ± 0.18 b	20.63 ± 0.59 cd
Bb03032	55.00 ± 0.11 abc	54.55 ± 0.16 a	18.64 ± 0.64 c
EABb04/01-Tip	45.00 ± 0.11 abcd	66.67 ± 0.17 a	19.11 ± 0.63 c
Bb64	40.00 ± 0.11 abcd	50.00 ± 0.19 ab	20.25 ± 0.59 c
Bb135	25.00 ± 0.10 bcd	40.00 ± 0.25 ab	20.80 ± 0.66 cd
Bb1022	30.00 ± 0.11 bcd	00.00 ± 0.00 b	21.00 ± 0.97 cd
Bb1025	35.00 ± 0.11 bcd	28.57 ± 0.18 b	20.29 ± 0.67 c
Naturalis® (strain ATCC74040-based bioinsecticide)	25.00 ± 0.10 bcd	22.22 ± 0.15 b	21.11 ± 0.68 cd
Control	00.00 ± 0.00 d	00.00 ± 0.00 b	24.60 ± 0.83 d

[a]Means (± SE) followed by the same letter within a column are not significantly different at $P < 0.05$ (Tukey's HSD test with Bonferroni correction for multiple testing).

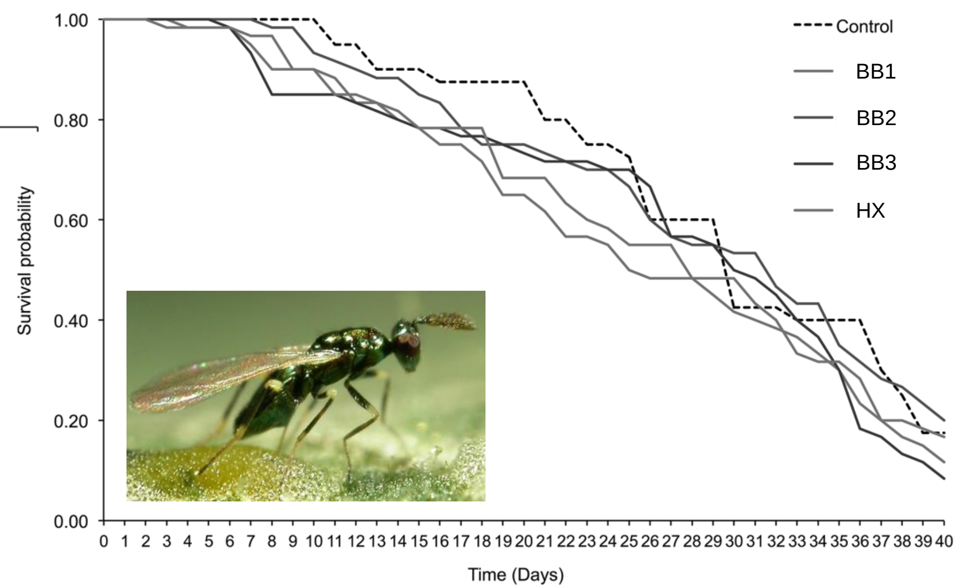
Vidal & Jaber 2015 Curr Sci

Endophyte colonization of *Vicia faba* by entomopathogenic fungi Isolates and life history of leafminer and parasitoid



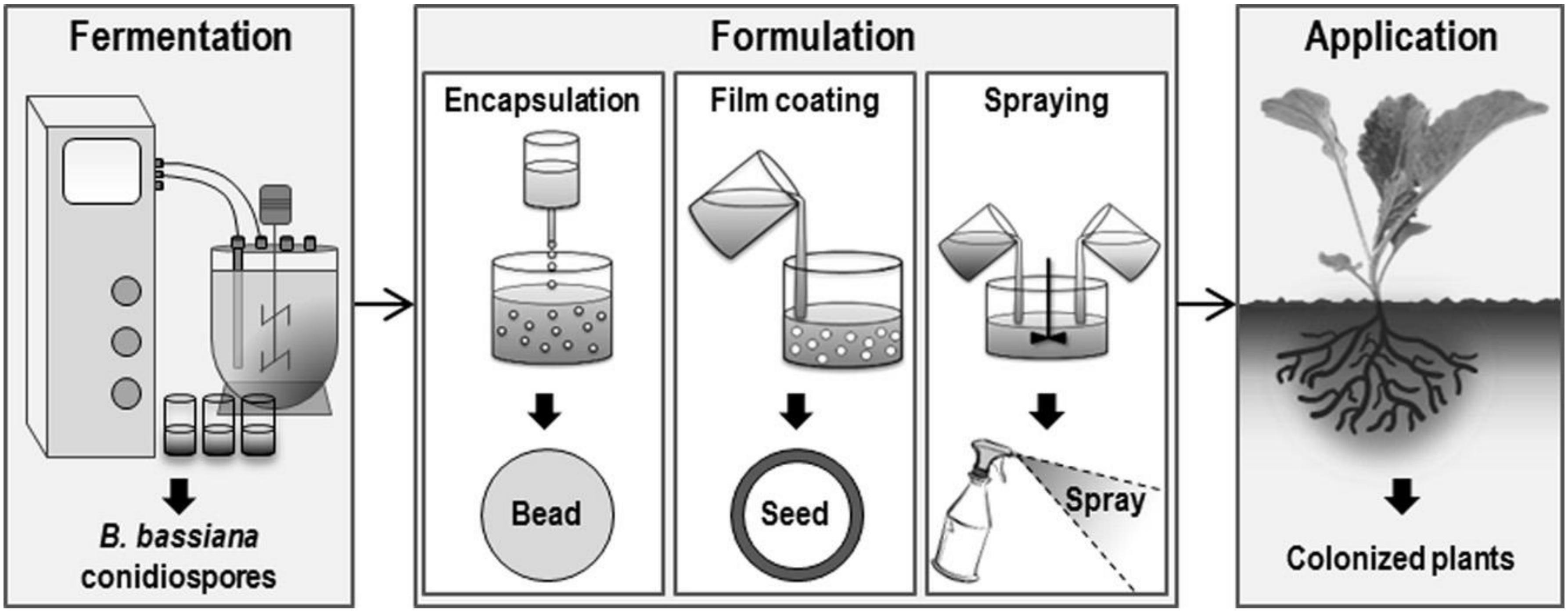
Beauveria bassiana (**BB**)
Hypocrea lixii (**HX**)

Diglyphus isaea



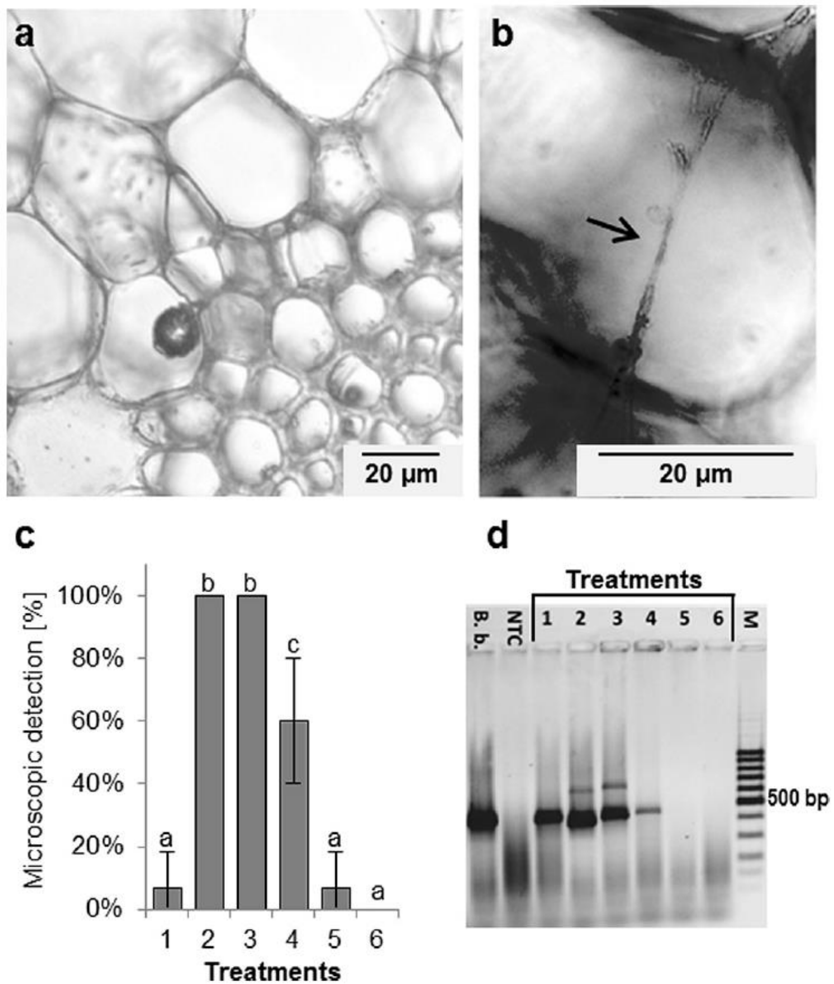
Akutse et al. 2014 PLOS ONE

Different strategies to enhance endophytic colonization of entomopathogenic fungi in plant tissues



Lohse et al. 2015 Biol Control

Influence of compositions from liquid formulations on endophytic colonization of oilseed rape leaves with *B. bassiana* after 14 days



Treatments:

1: water, *B. bassiana* (BB) - 2: Triton X-114 (Tri), sugar beet molasses (M), Titanium oxide (Td), BB
3: TRI, M, BB - 4: Tri, TD, BB - 5: M, TD, BB - 6: TRI, M, TD

Lohse et al. 2015 Biol Control

Pros and cons of using endophytic fungi

+ Specific isolates may target specific pest species

+ Entomopathogenic fungi are also working as endophytes

+ Mass production possible; costs therefore reasonable

+ Combination with other BCAs possible

- Colonization of all plant tissues not a trivial task

- Metabolite production in planta of concern

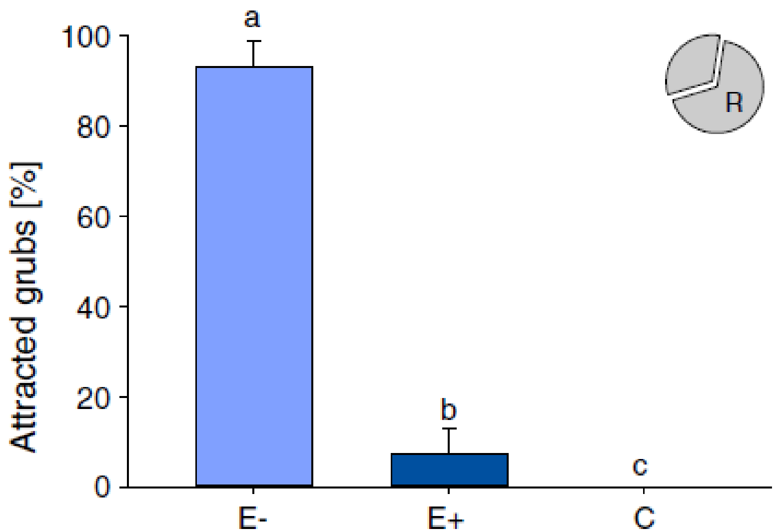
- Formulation issues not yet properly addressed

Use of multitrophic interactions

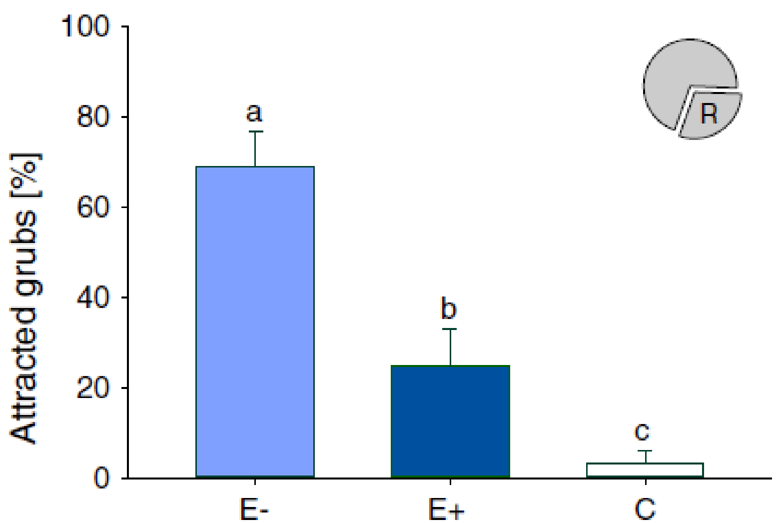
What do we know about multitrophic interactions?

- HIPVs produced by plants upon attack by herbivore pests
- Many of these HIPVs herbivore species specific
- Know to increase parasitism rates
- Cultivars differ in HIPV bouquets

Response of *Costelytra zealandica* grubs to grass root volatiles in a four-arm olfactometer



Roots accessible

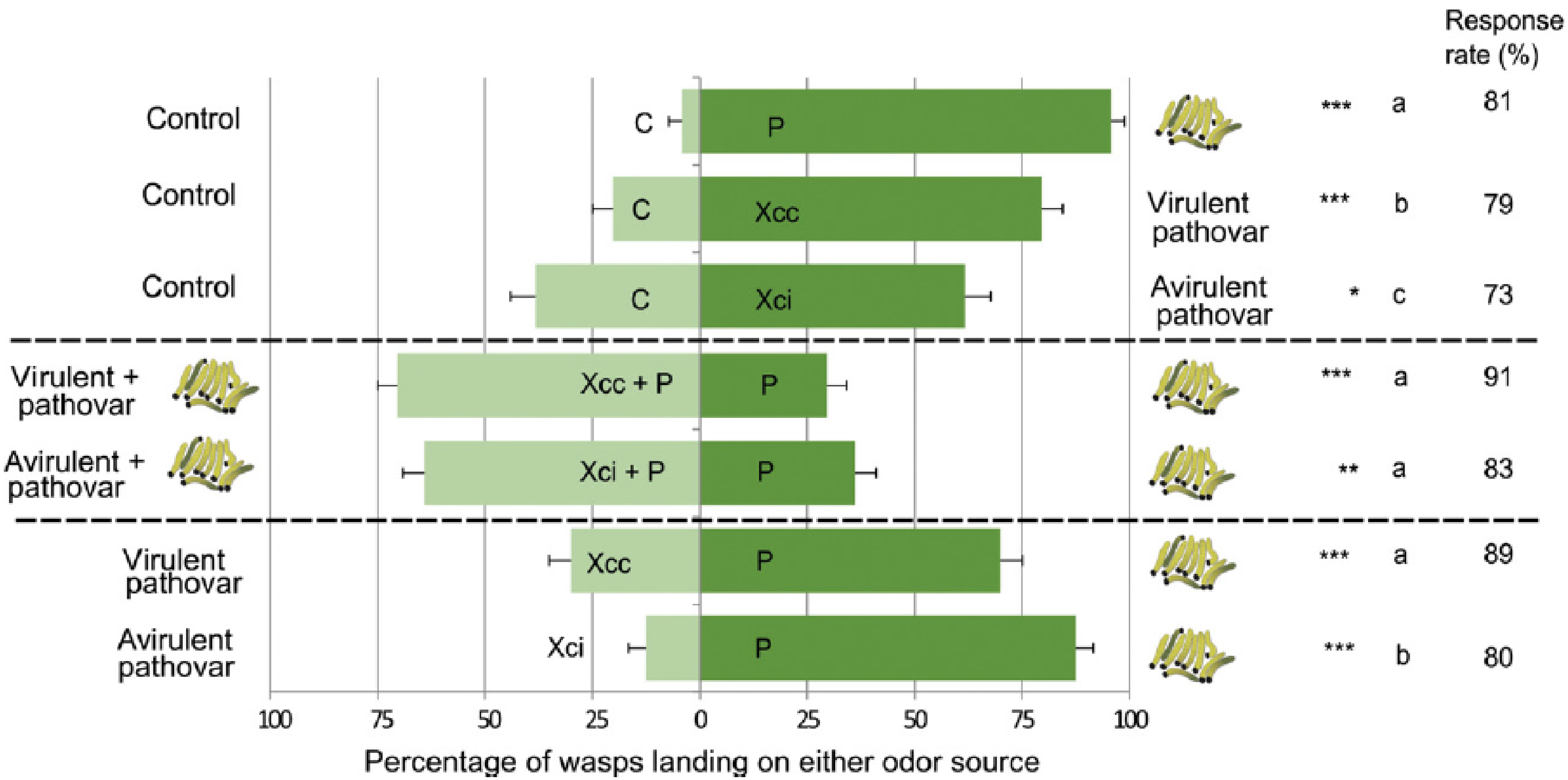


Roots not accessible

Rostas et al. 2015 Oecologia

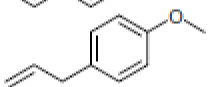
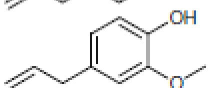
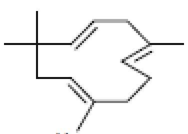
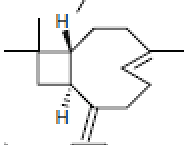
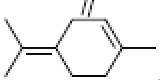
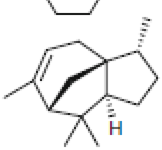
Response rate of *Cotesia glomerata* in wind tunnel to *P. rapae* larvae on healthy and bacterium inoculated plant

Xanthomonas campestris pv. campestris



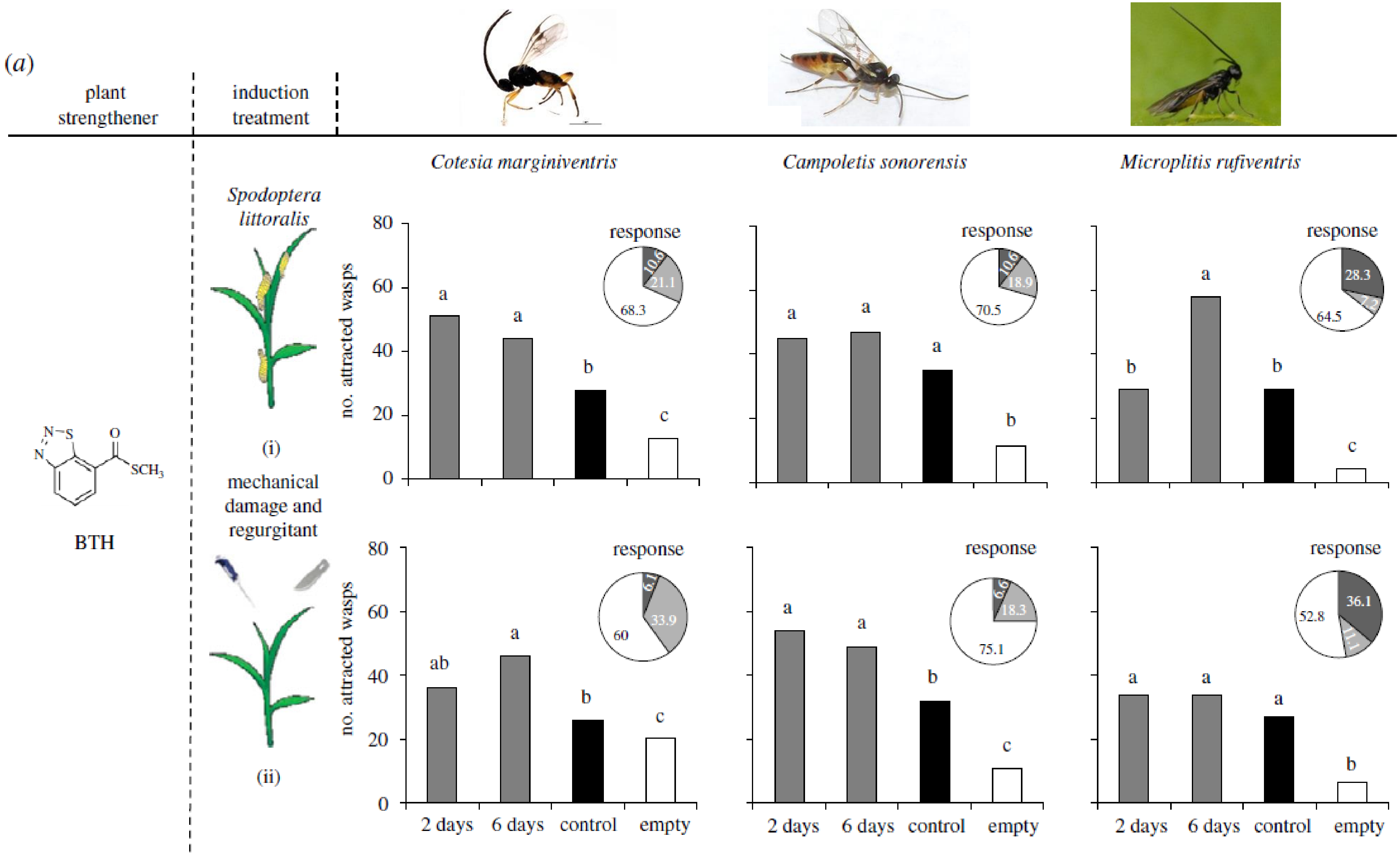
Ponzio et al. 2016 Funct Ecol

Key compounds involved in stemborer control emitted by intercrop and trap plants in push–pull cropping systems

Pull semiochemistry					
Structure	**Compound**	**Source**	**Method for ID**	**Target**	**Activity**
	Octanal	Maize (*Zea mays*)	GC-EAG, GC-MS, co-elution with standards	Stemborer moths (adults)	Host cue attractant
	Nonanal	Napier grass (*Pennisetum purpureum*)			
	Naphthalene				
	4-allylanisole				
	Eugenol				
	Linalool				
Push semiochemistry					
*† 	(*E*)-Ocimene	*Melinis minutiflora*	GC-EAG, GC-MS, co-elution with standards	Stemborer moths	Repellent
*† 	(*E*)-4,8-dimethyl-1,3,7-nonatriene	†*Desmodium* spp.		**Cotesia sesamiae*	Attractant
	Humulene				
	β-Caryophyllene				
	α-Terpinolene				
† 	α-Cedrene				

Khan et al. 2016 J Chem Ecol

Enhancing the presence and efficacy of native biological control agents by applying plant strengtheners



BTH: benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester

Sobhy et al. 2014 Phil Trans R Soc B

Pros and cons of using multitrophic interactions

+ Compounds specifically attract specific parasitoids or predators

+ Non-target effects minimal

- Effects on pest population levels not clear

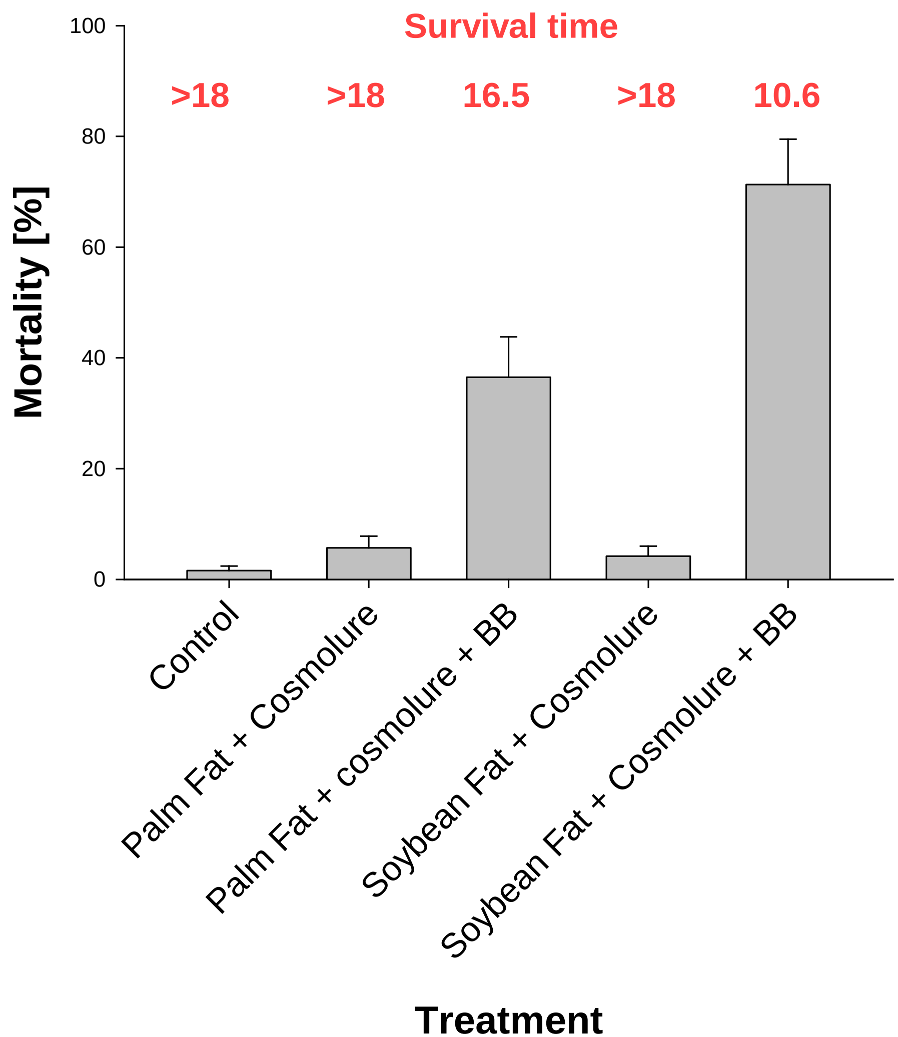
- Application costs a challenge

- Plant breeders not willing to step in

Enhancing the efficacy of native biological control agents by combining different agents

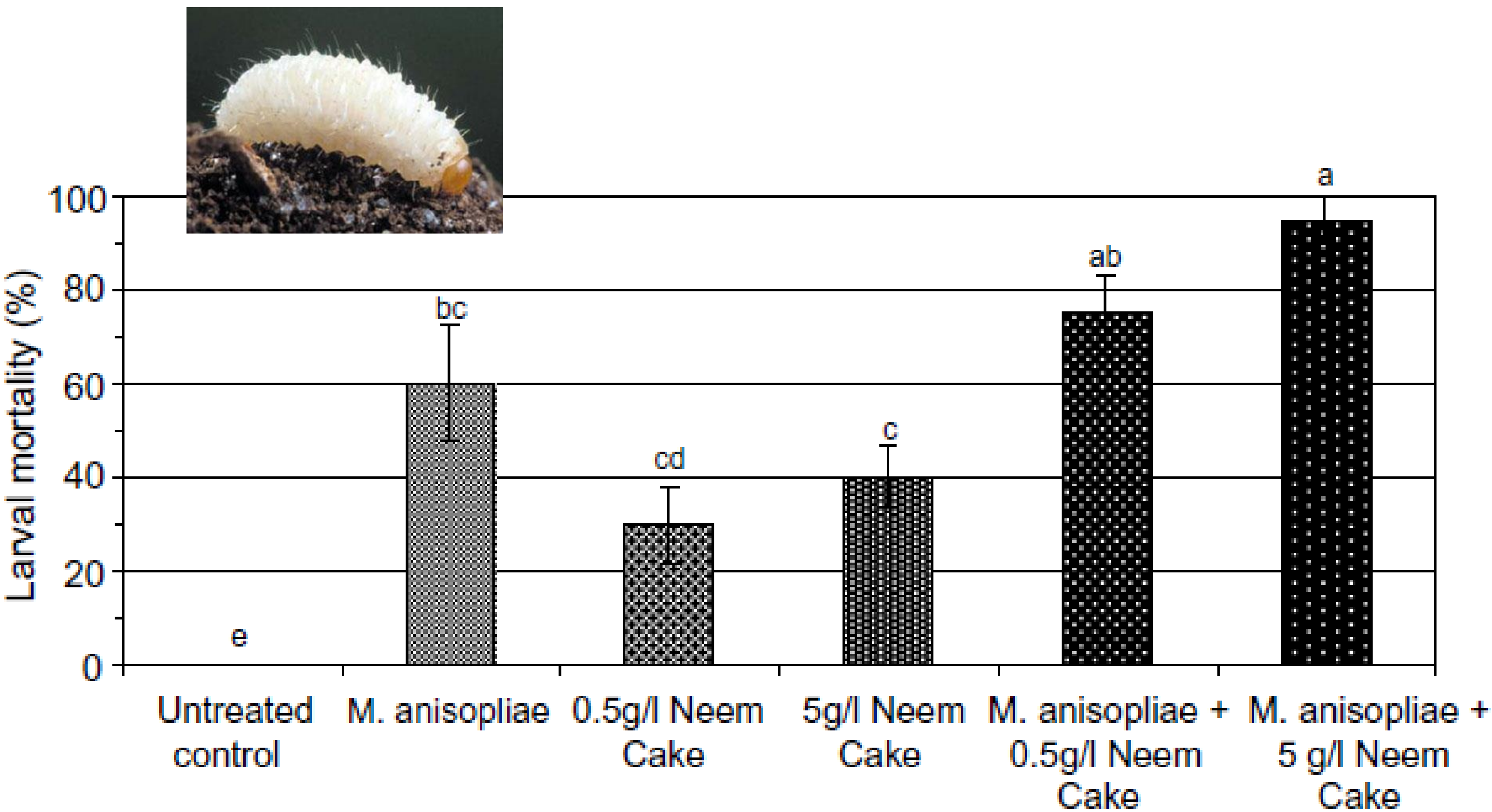
Combined use of a pheromone and an entomopathogenic fungus for banana weevil control

Cosmopolites sordidus



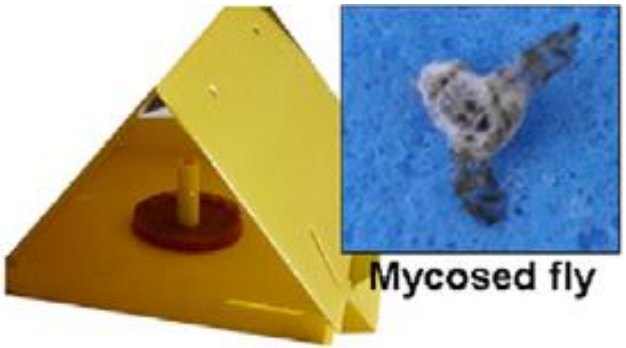
Lopez et al. 2014 Ent Exp Appl

Mortality of BVW larvae in potted Euonymus plants treated with combinations of Neem cake or *Metarhizium sp.*

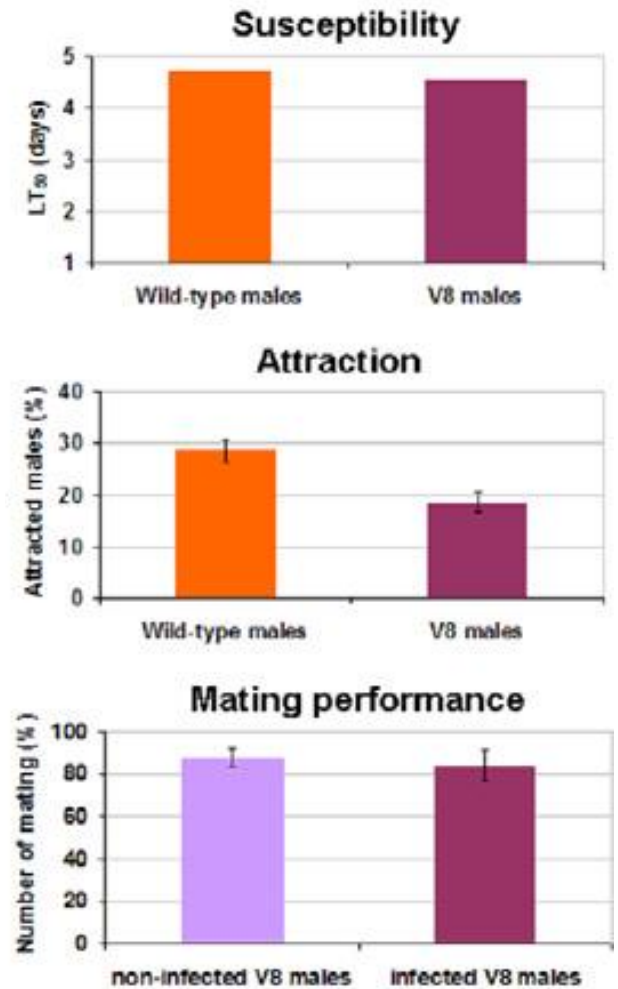


Shah et al. 2008 Biol Control

Combination of an attractant contaminant device containing an entomopathogenic fungus for Medfly control using sterile males



Attractant Contaminant Device



C. capitata V8 strain

San Andres et al. 2014 Biol Control

Pros and cons of BCA combinations

+ Synergistic or additive effects possible

+ Non-target effects minimal

- Problems when it comes to registration

- Application costs too high (?)

- Potential combinations yet only marginally explored

Development of an “attract and kill” strategy for soil dwelling pests

Most important common targets for new biocontrol products identified in arable, vegetable, and perennial crops in European

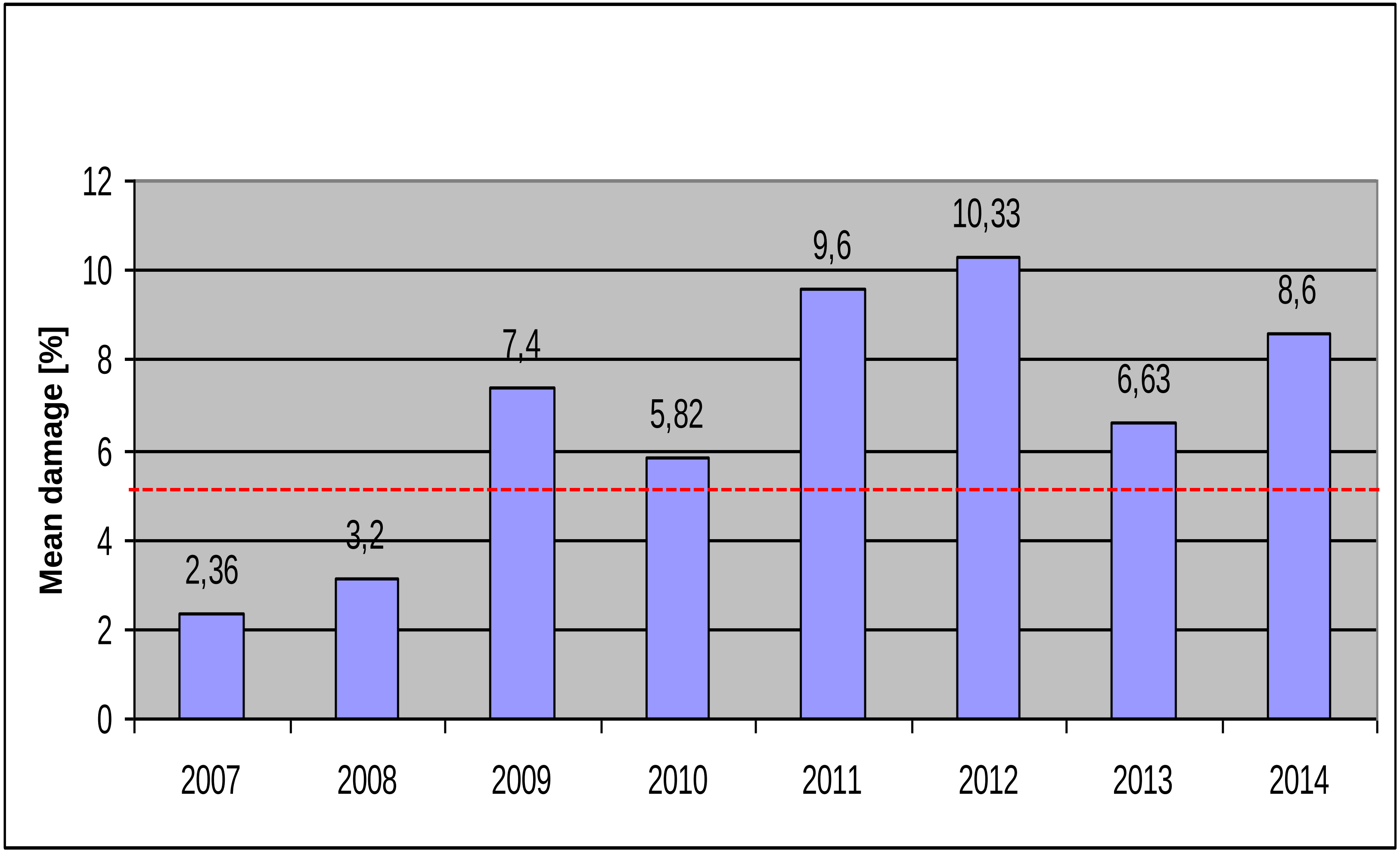
Pest	Crop(s)	Control solution	Available
Wireworms	Potato, maize	Entomopathogenic fungi	No - *yes*
Pollen beetle	Oilseed rape	Entomopathogenic fungi; entomopathogenic nematodes	No
Weevils	Oilseed rape	Parasitoids	No
Flea beetles	Oilseed rape	Entomopathogenic nematodes	No
Root flies	Brassicas	Beneficials	(Yes)
Drosophila suzukii	Stone & soft fruits	None	None

Lamichhane et al. 2017 Pest Manag Sci

Damage on potato tubers caused by wireworms



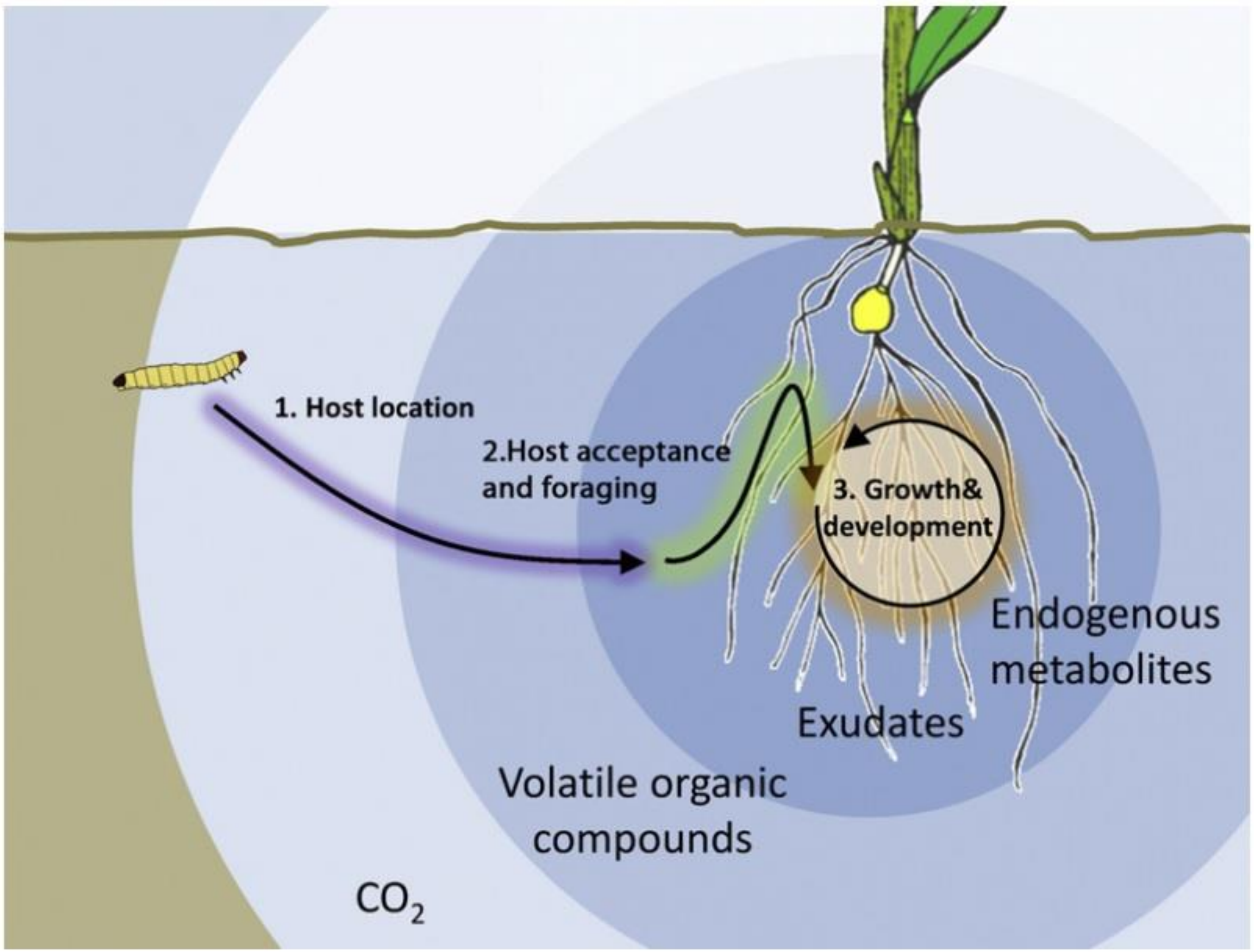

Mean wireworm damage in organic potato fields in Lower Saxony and Bavaria



The problem

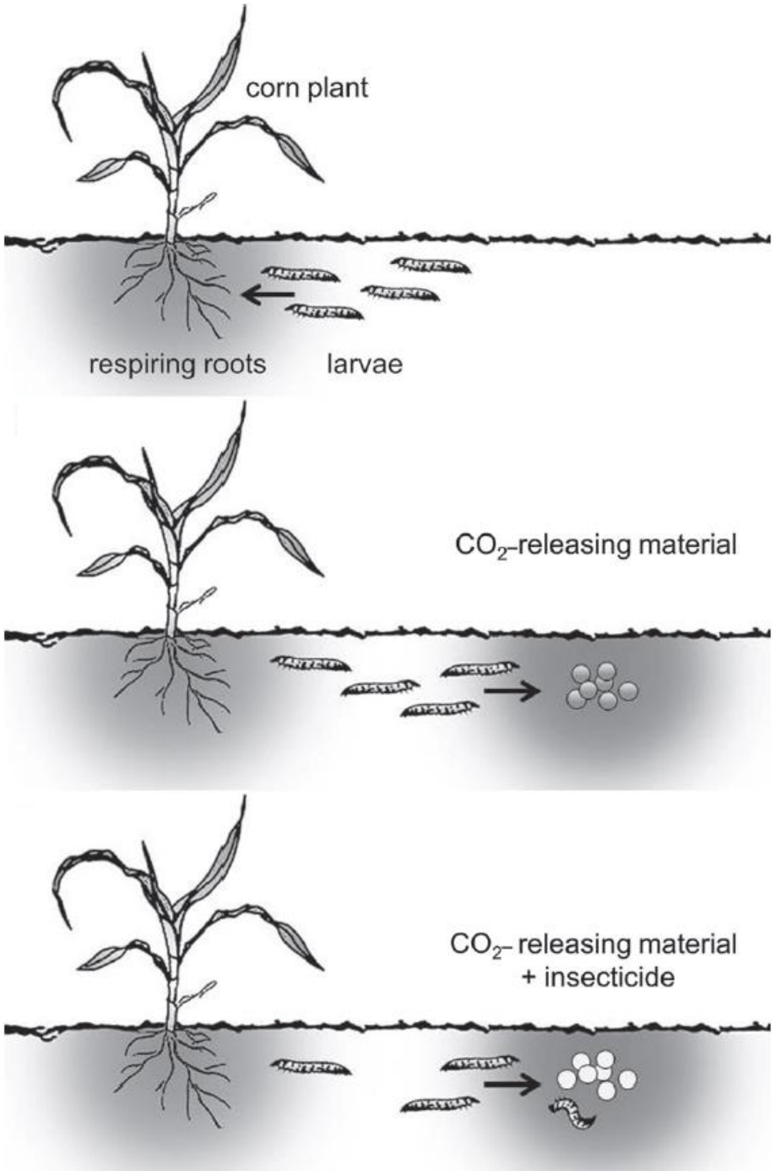
- Increasing wireworm damage reported in different crops (arable, vegetables, greenhouse)
- Control of wireworm species difficult because of
  - “Old” insecticides banned (i.e. Lindan)
  - Phasing out of efficient a.i.$_s$ (Neonicotinoids, Fipronil (?))
  - Application techniques mostly not suitable
  - Patchy distribution

Orientation cues of (most) soil dwelling larvae



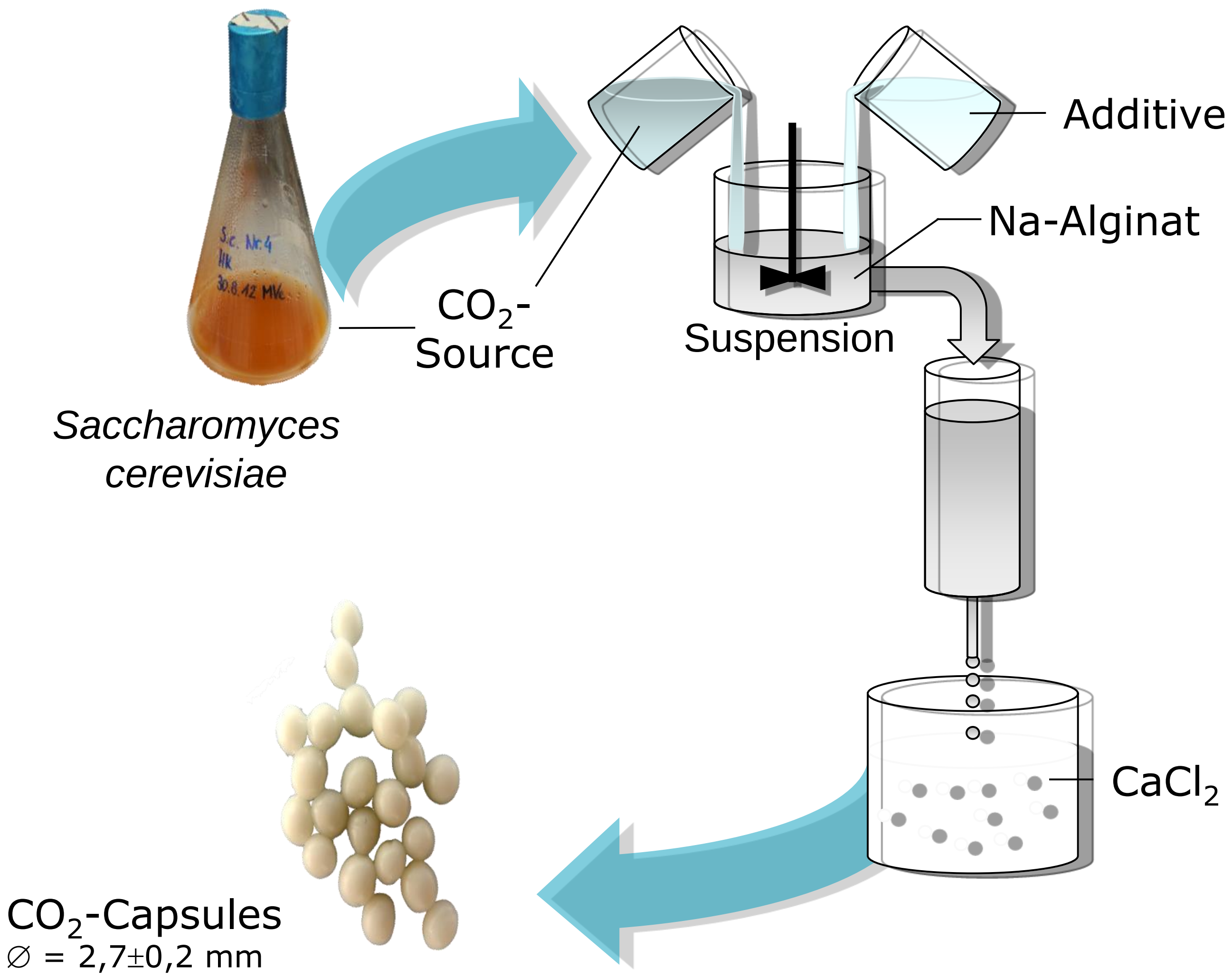
Erb et al. 2013 Adv Ins Phys

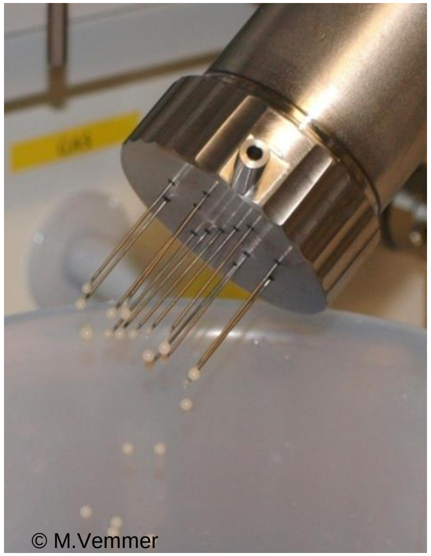
Orientation of soil-dwelling larvae towards CO_2 gradients of growing maize plants



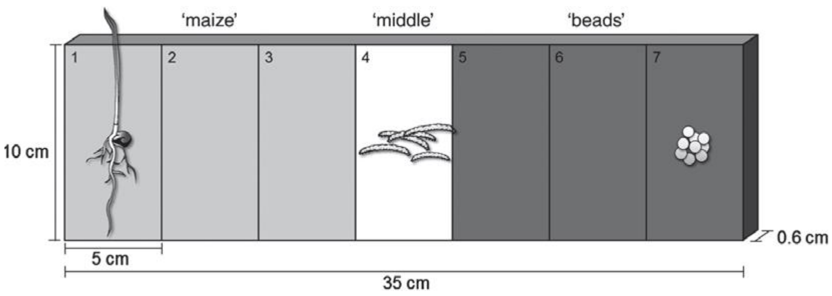
Vemmer et al. 2016 Pest Manag Sci

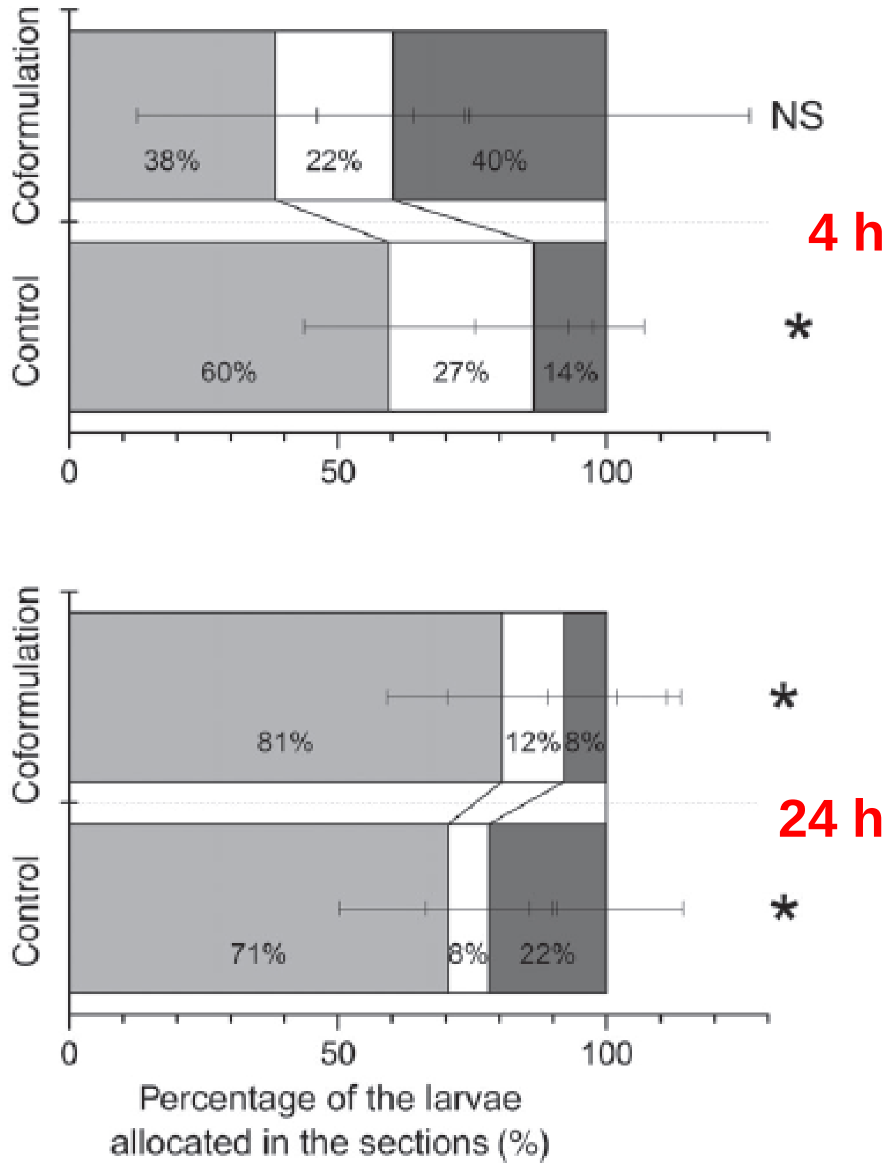
Production of CO_2 emitting capsules





Orientation of western corn rootworm larvae when exposed to maize roots or CO_2 releasing compounds





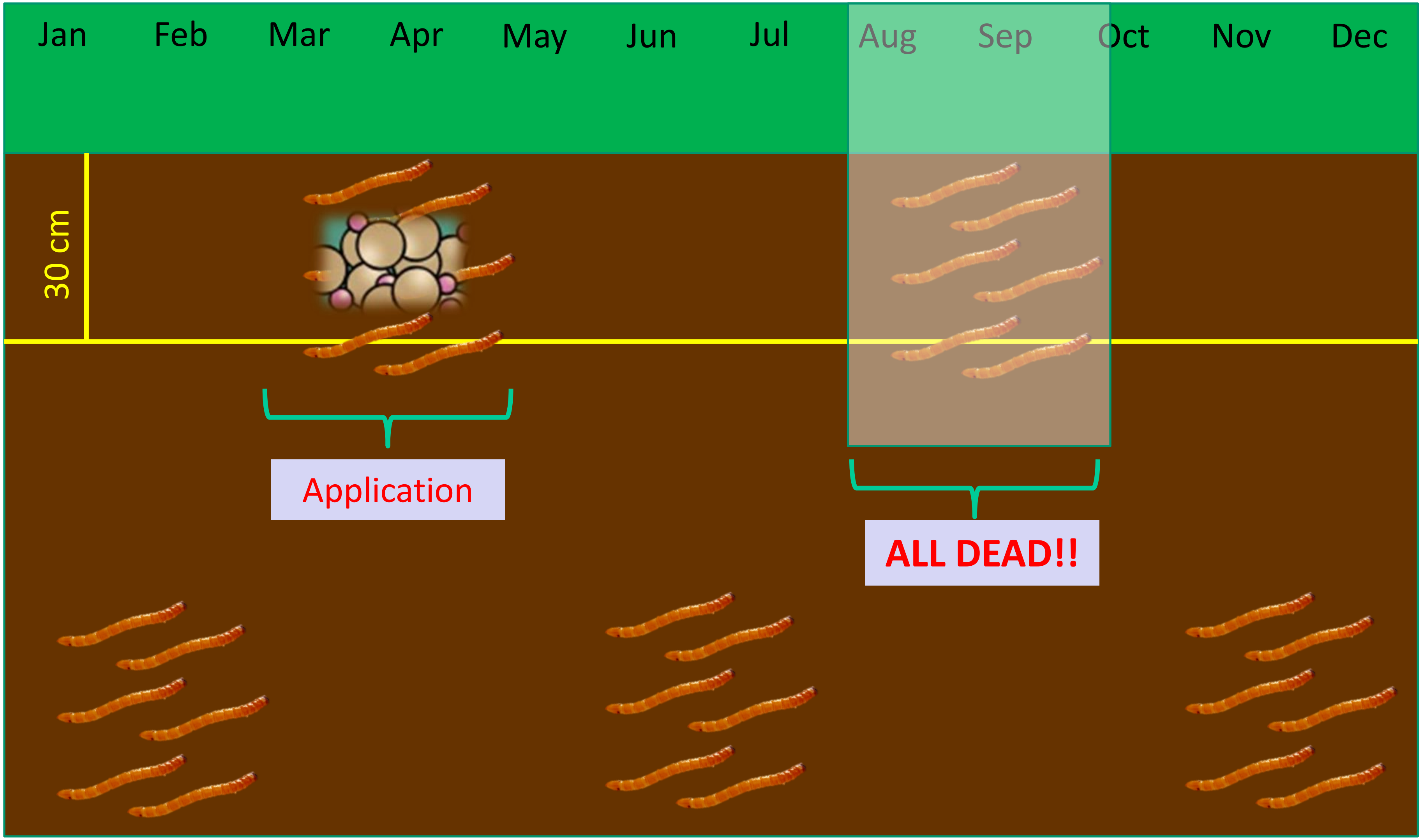
Vemmer et al. 2016 Pest Manag Sci

Addition of a kill-component

- Insecticides (*Force 1.5G*)
- Spinosad
- Neem
- Entomopathogenic fungi

  (*Metarhizium brunneum* [ART 2825])

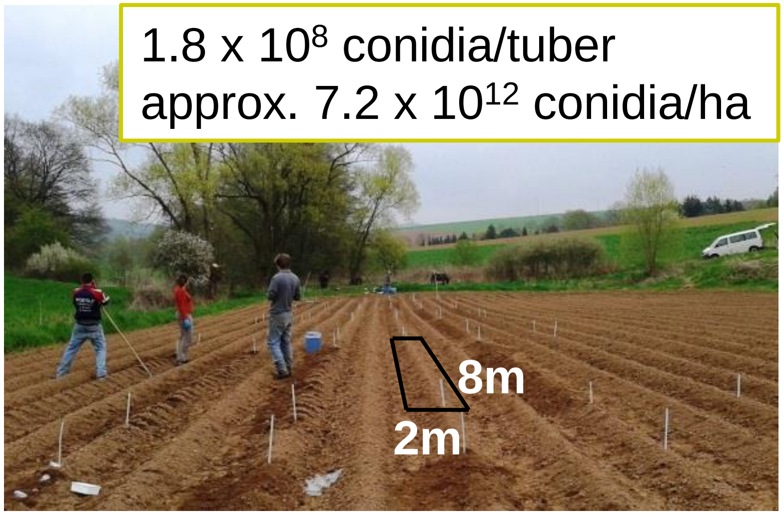
Vertical movement of wireworms and window of vulnerability



Experimental set-up

- Fields in different federal states (2013-15)
- Conventional, organic farming
- Randomized block design
  - 6 to 8 reps (EPPO guideline)
  - Plot: 1 potato row (8 m length)
  - Untreated buffer rows

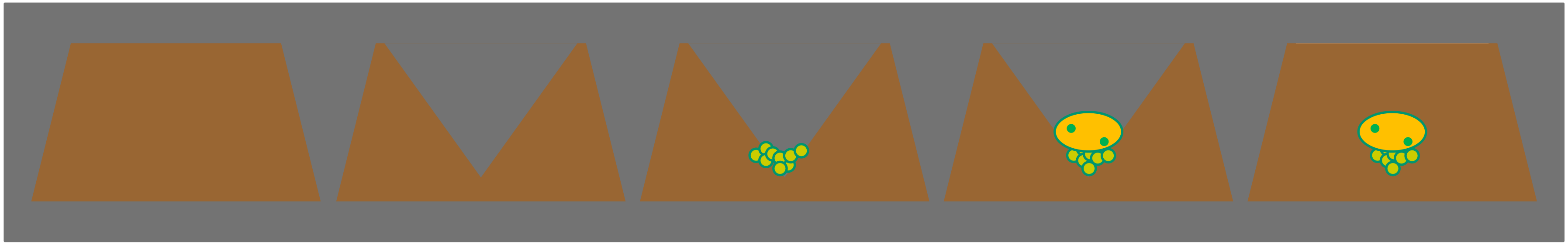
1.8 x 10^8 conidia/tuber
approx. 7.2 x 10^{12} conidia/ha



<u>Treatments</u>

– **Control**
– *M. brunneum* - suspension
– *M. brunnem* - beads
– CO_2-beads
– Attract + Kill – beads
– Firponil (Goldor Bait®)

- Applications
  - Spot (Beneath, between)
  - Band (Beneath)



Every beginning is difficult: Spoon-application-technology

Field tests using co-formulated A&K compounds

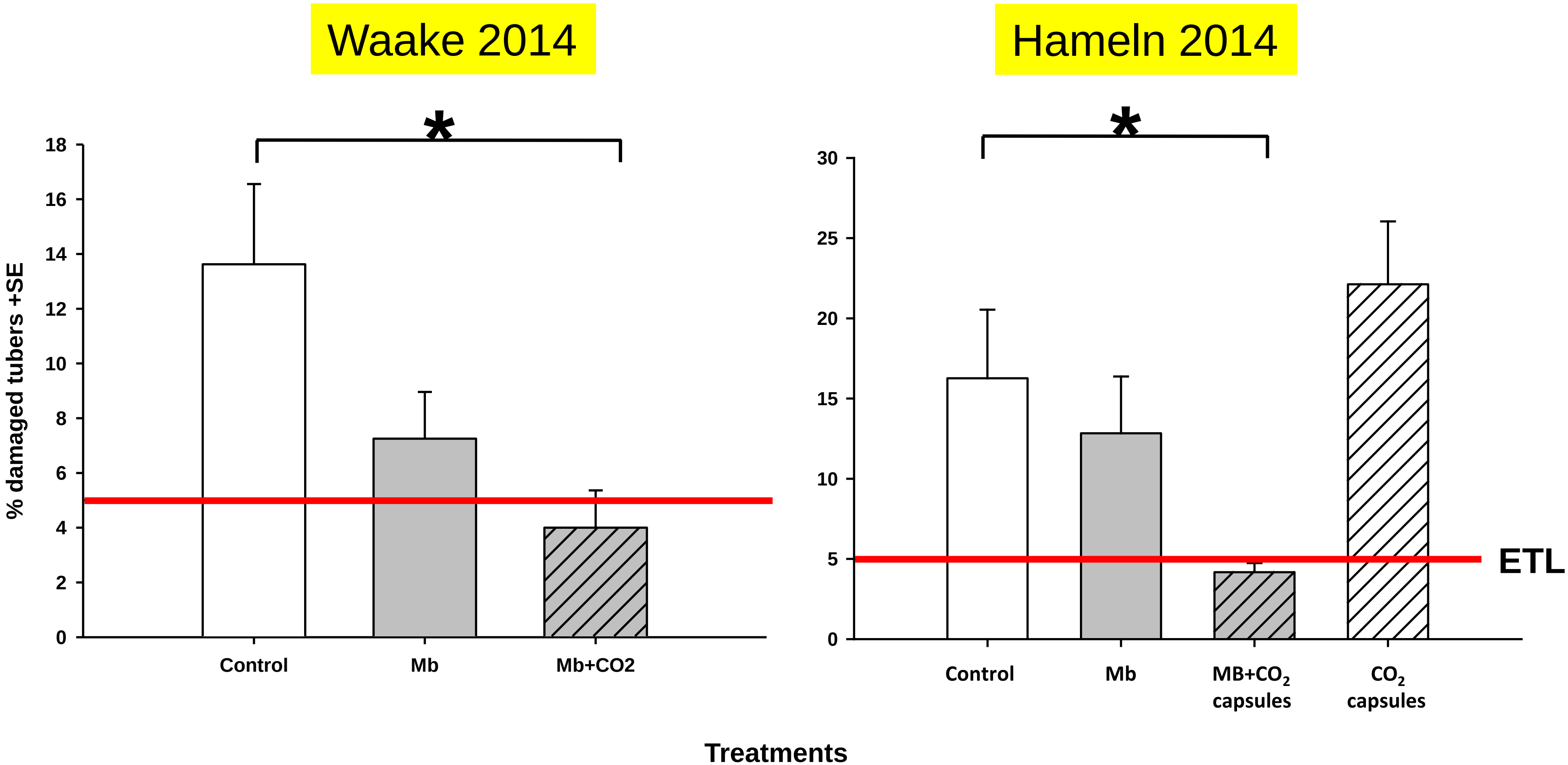
AK-capsules

AK-granules

Rheinland-Pfalz
DIENSTLEISTUNGSZENTREN LÄNDLICHER RAUM

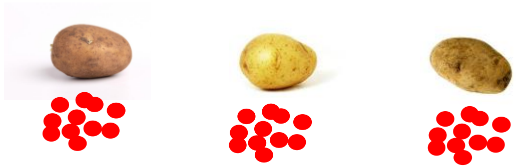
Landwirtschaftskammer Niedersachsen

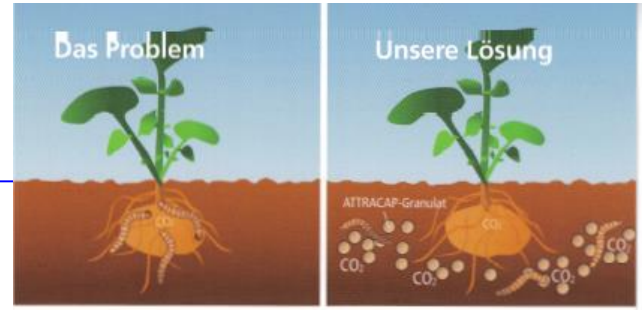
Damage assessment on potato tubers: *Metarhizium brunneum* as the killing agent



Kruskal-Wallis: $H_{2,\,24} = 7.09$ $P < 0.05$

Kruskal-Wallis: $H_{3,\,31} = 9.81$ $P < 0.05$





ATTRACAP

ATTRACAP von BIOCARE ist ein **innovatives biologisches Insektizid** zur Bekämpfung von **Drahtwürmern**. Es ist zugelassen nach Artikel 53 für Notfallsituationen (01.03.2016–28.06.2016). Im Kartoffelanbau haben wirtschaftliche Verluste durch Drahtwurmbefall erheblich zugenommen. Drahtwürmer, die Larven des Schnellkäfers, fressen tiefe Löcher in die Kartoffeln, die dadurch teilweise nicht mehr vermarktet werden können.

Die Wurzeln der Kartoffeln geben CO_2 ab und locken damit die Drahtwürmer an. Die **Attract-and-Kill-Strategie** von **ATTRACAP** nutzt dies. Das Produkt erzeugt über ca. 4 Wochen CO_2, wodurch die Drahtwürmer angelockt werden. Neben der CO_2-Quelle befindet sich im Granulat der natürlich vorkommende, insektenabtötende Pilz *Metarhizium brunneum*. Dieser wächst aus dem Granulat aus. Durch den Kontakt mit den ausgewachsenen Sporen infizieren sich die Larven und sterben nach einigen Tagen ab.

Anwendung

Befallsstärke:	Niedriger bis mittlerer Befall
Wirkstoff:	*Metarhizium brunneum*
Ausbringmenge:	30 kg je Hektar
Ausbringmethode:	Granulatstreuer (JKI-geprüft)

Pflanzenschutzmittel vorsichtig verwenden. Vor Verwendung stets Etikett und Produktinformationen lesen!

BIOCARE-Forschungsförderung durch:

BIOCARE Gesellschaft für Biologische Schutzmittel mbH
Dorfstr. 4 ▪ D-37574 Einbeck ▪ Tel. (0 55 62) 95 05 78-0
Fax (0 55 62) 95 05 78-9 ▪ E-Mail: info@biocare.de ▪ www.biocare.de

1107/2009 EG Article 53
Emergency situations in PP

Summary of efficacy data from independent farmer field trials 2016

Land-wirt	Region	Befall im Vorjahr	Bekäm-pfung im Vorjahr	Kultur im Vorjahr	Bodentyp	Beregnung	Anzahl befallener Knollen / 100 Knollen		WG	Bemerkung
							unbehandelte Fläche	behandelte Fläche	%	
1	Vorderpfalz / Rheinland-Pfalz	schwach	ja	Zuckerrüben, Sommergerste	sandiger Lehm	ja	0	0	-	nicht bewertet
2	Vorderpfalz / Rheinland-Pfalz	schwach	ja	Sommerzwiebel	uL	ja	3	3	0	nicht bewertet, da unter dern ökonomischen Schadensschwelle
3	Rheinhessen / Pfalz	schwach	ja	Kartoffel	sL	ja	40	10	75	
4	Rheinland-Pfalz	schwach	ja	Getreide	sL	ja	keine	0	-	nicht analysierbar, da keine Kontrolle vorhanden
5	Rheinland-Pfalz	mittel	nein	Karotten	lS	ja	23	11	52	
6	Rheinland-Pfalz	unbekannt	nein	Winterweizen	sL	ja (über-schwemmt)	keine	3,5	-	nicht analysierbar, da keine Kontrolle vorhanden
7	Vorderpfalz / Rheinland-Pfalz	stark	nein	Zwiebeln	sL	ja	keine	6	-	nicht analysierbar, da keine Kontrolle vorhanden
8	Worms / Pfalz	nein	nein	Weizen	sL	ja	3	1	67	nicht bewertet, da unter dern ökonomischen Schadensschwelle
9*	Wittlich, Rheinland-Pfalz			Raps	sL	nein	20	0	100	
10	NRW	mittel	ja	Wintergerste	sL	ja	30	15	50	
10	NRW	mittel	ja	Wintergerste	sL	ja	30	4	87	

Ø	**72,8**
SD	**21,8**

Conclusions

- New technologies provide opportunities targeting organisms not yet controlled
- The potential for innovative formulations, combining different BCAs has not yet been fully exploited
- Artificial Co_2-gradients are attractive for soil dwelling insects
- Co_2-releasing capsules offer new opportunities to increase the efficacy of biocontrol agents
- The attract & Kill-strategy can be extended to other target organisms

Project funds for „A & K“ since 2012

FH Bielefeld
University of
Applied Sciences

ÖKORING

INBIOSOIL
Innovative biological products for soil pest control

Bundesministerium für
Ernährung, Landwirtschaft
und Verbraucherschutz