Predicting Persistence of Microbial Biocontrol Agents

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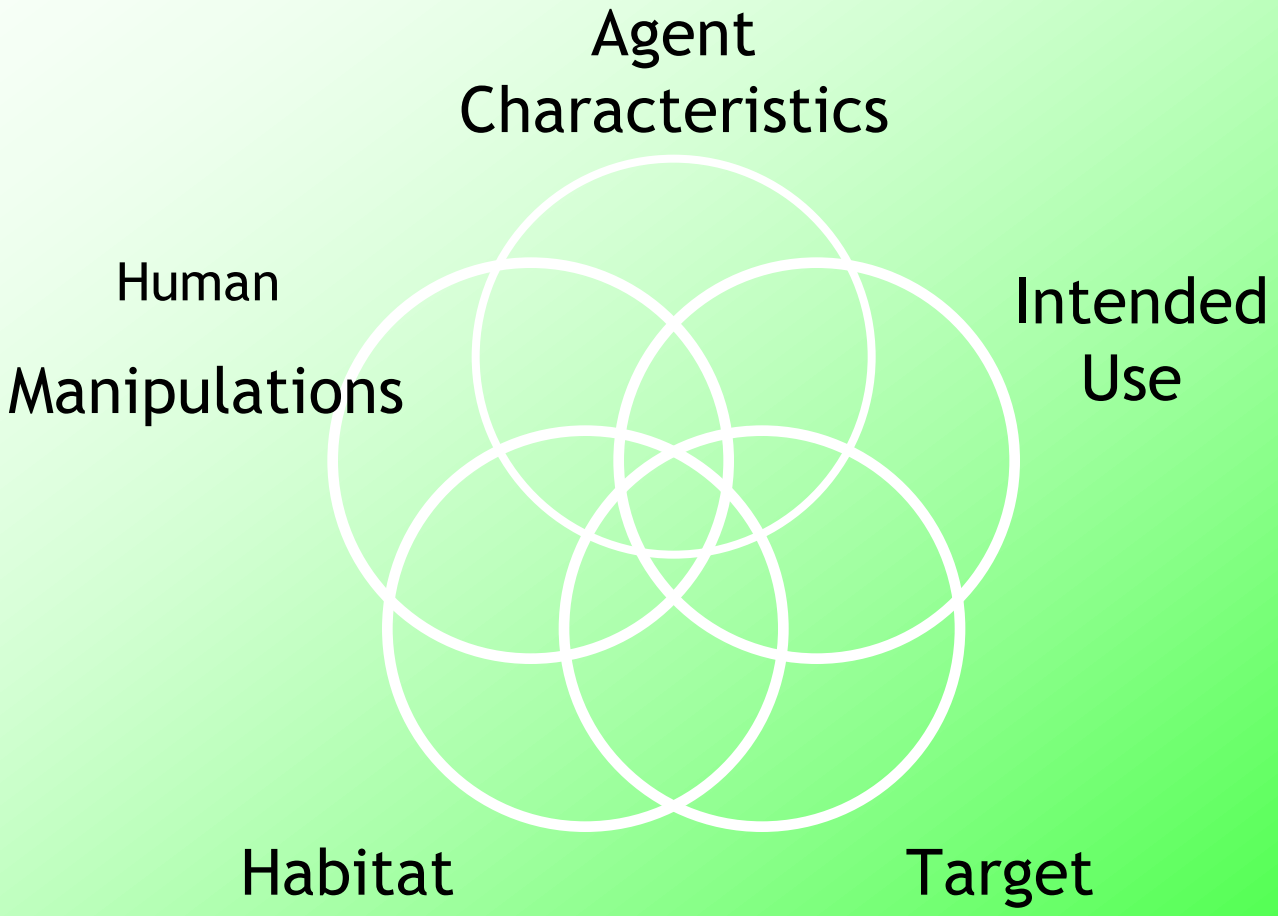
Beauty is in the eye of the beholder

Persistence is in the eye of the user

Persistence of an Agent is determined by …



The intended use pattern affects persistence

… by defining the desired characteristics of the microbial agent

- **Innundative use** seeks short-moderate term establishment
  (under FIFRA, registration = $$ = commercial = repeat use (sales) … for ROI)
- **Classical biocontrol** seeks long term (permanent?) establishment, and spread, of agent after inoculation of agent
- **Induction of Systemic Plant Resistance**
- **Multifunctional Endophyte Plant Symbioses**

The intended use pattern affects persistence

… by defining the desired characteristics of the microbial agent

- **quantity**
  - efficacious amount
  - number of applications
- **specific mode of action**
  - outright pathogenesis, mycoparasitism
  - competitive exclusion
  - plant systemic resistance induction
  - plant hormonal changes
  - multiple mechanisms
- **degree and timing of control**
  - short term catastrophic mortality
  - long term population/disease reduction thru chronic effect
  - prophylactic vs. post outbreak use

The target affects persistence

Invertebrates: transient; often mobile; some multigenerational; sometimes cryptic; damage crop quickly.

Plants (weeds): stationary, longer lived, slower, more subtle crop damage

Plant Pathogens: many endobionts, others in soil; some are vectored; variable rates of pathogenesis

… these define the desired characteristics of the microbial agent and thus its necessary persistence.

The transiency of the habitat affects persistence

- **annual crop**
  (very transient; catastrophic end)
- **biennial, perennial crop**
  (more prolonged – 2 seasons with interruption – but with catastrophic end)
- **long lived crops (orchards)**
  (>2 years, some stability, with periodicity of crop and pest)
- **natural habitats**
  (long term stability, maybe with seasonality)

The nature of the habitat affects persistence

On the Phylloplane

- UV-A/UV-B radiation
- rainfall
- phylloplane pH
- phylloplane chemistry
- phylloplane microbiota
- temperature
- target host population

The nature of the habitat affects persistence

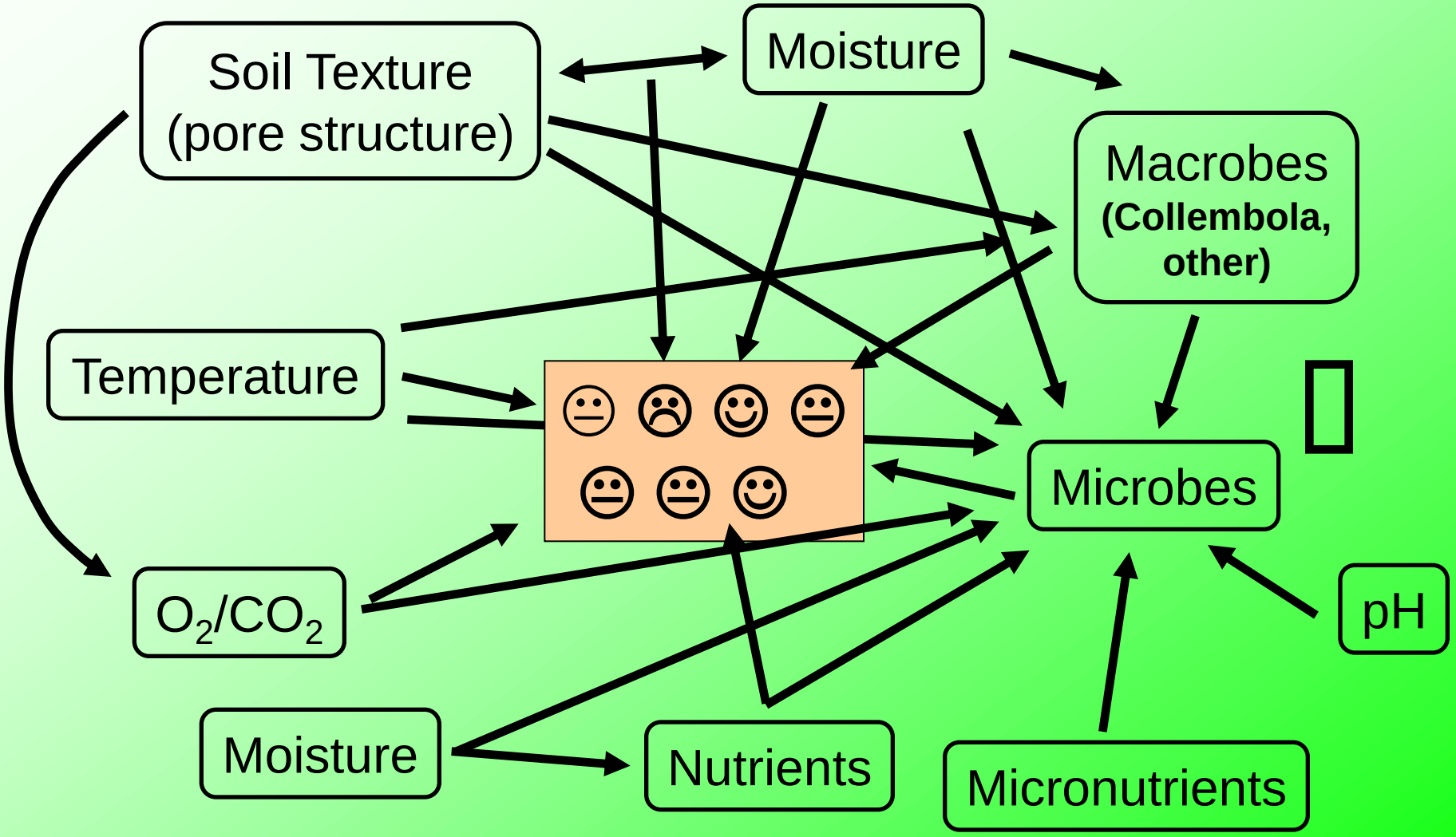
In the Soil

- moisture
- temperature
- microbiota
- macrobiota
- soil chemistry (direct & indirect)
- soil texture
- O_2 / CO_2
- target host population

Abiotic factors inter-related and affect both infectivity and persistence

Biotic and abiotic factors interact

Interaction is dynamic and variable over time



The nature of the habitat affects persistence

In Aquatic Habitats

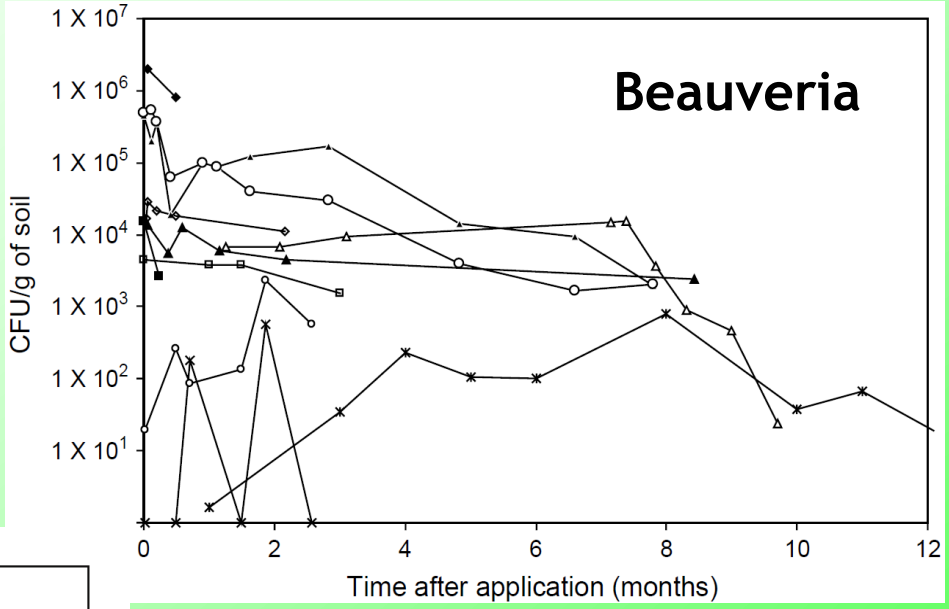
- temperature
- chemicals (e.g., organic pollutants)
- physical sequestration (sedimentation, removal by currents)
- micro fauna
- macro fauna
- host population

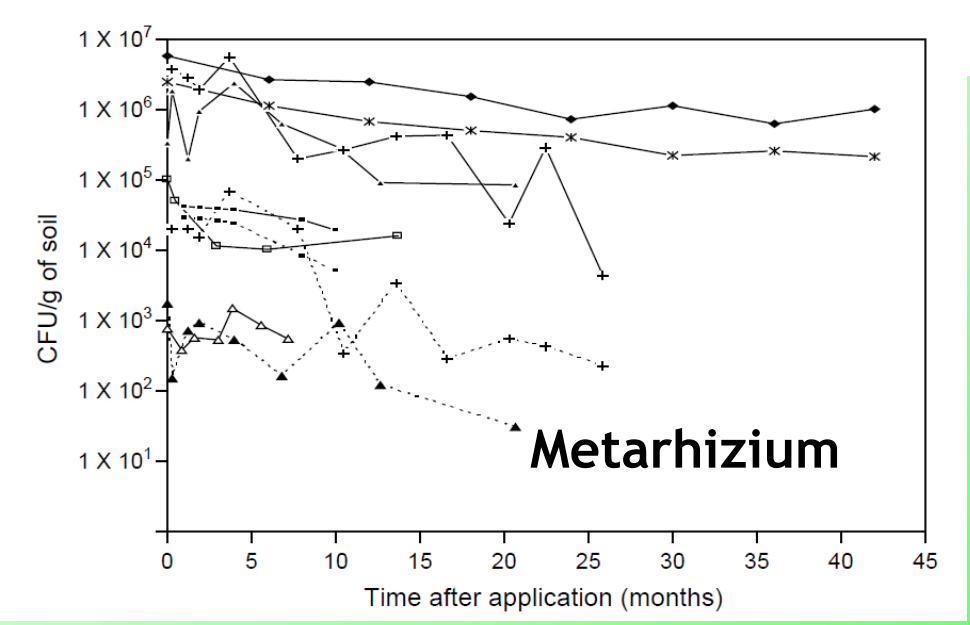
How can we predict persistence? … realistically

- can we generalize from the pathogen group ?

Inundative applications decline with time

Sometimes quickly, sometimes slowly, with considerable variability

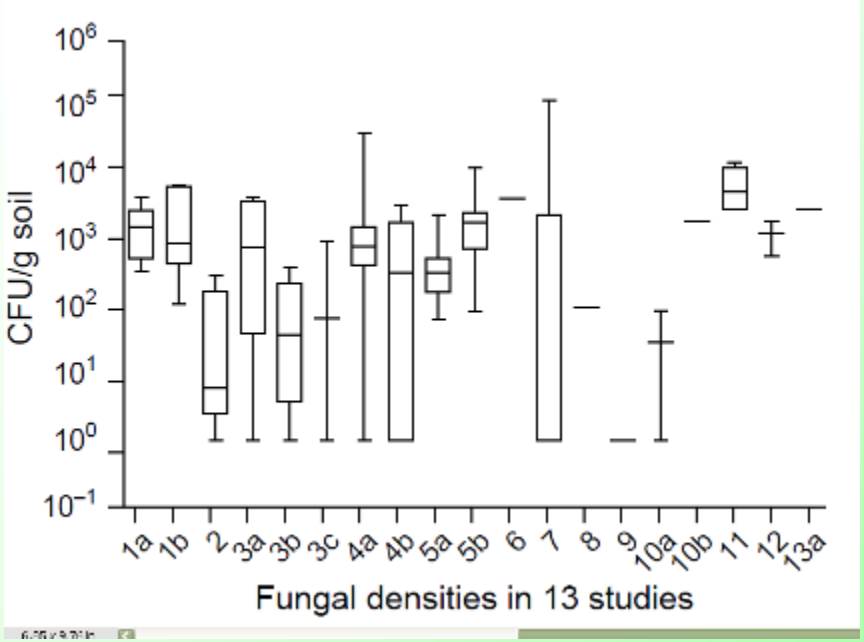




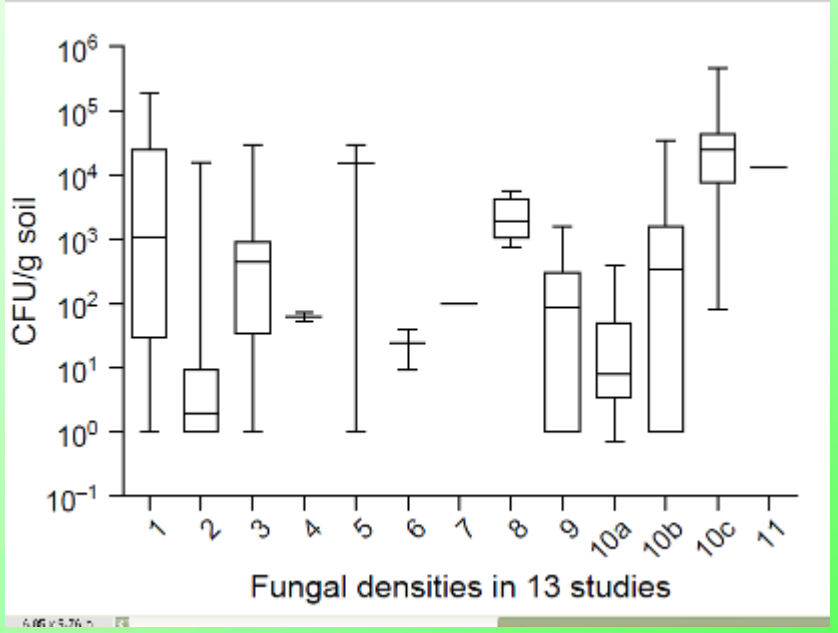
Critical EPF levels for insect control are **10^5-10^6 CFU/ g** soil vs *economic* application rates of **$2x10^4$-$1x10^6$ CFU/g**.

Scheepmaker and Butt 2010 Biocontrol Sci & Technol

“EPF” remain at low levels in nature.



Metarhizium spp.



Beauveria bassiana

In most cases these levels are too low to be useful, or ‘dangerous’

Critical EPF levels for insect control are **10^5-10^6 CFU/ g** soil
vs *economic* application rates of **$2x10^4$-$1x10^6$ CFU/g.**

Scheepmaker and Butt 2010 Biocontrol Sci & Technol

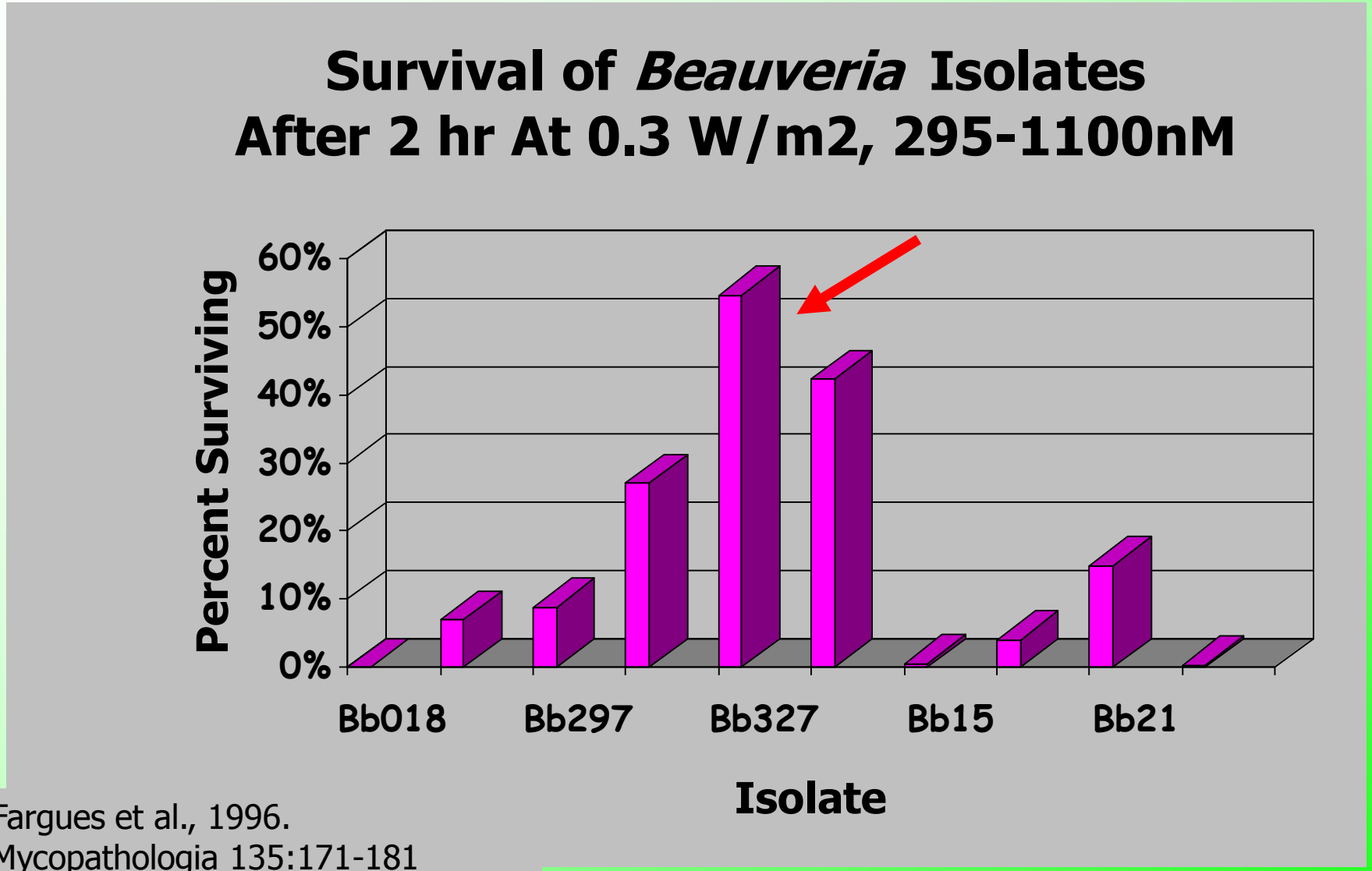
Can we generalize from the pathogen group ?

Caveat emptor

There are differences among isolates.

Persistence in the face of specific environ-
mental variables is after all a
phenotypic character

Tolerance to Ultraviolet Radiation

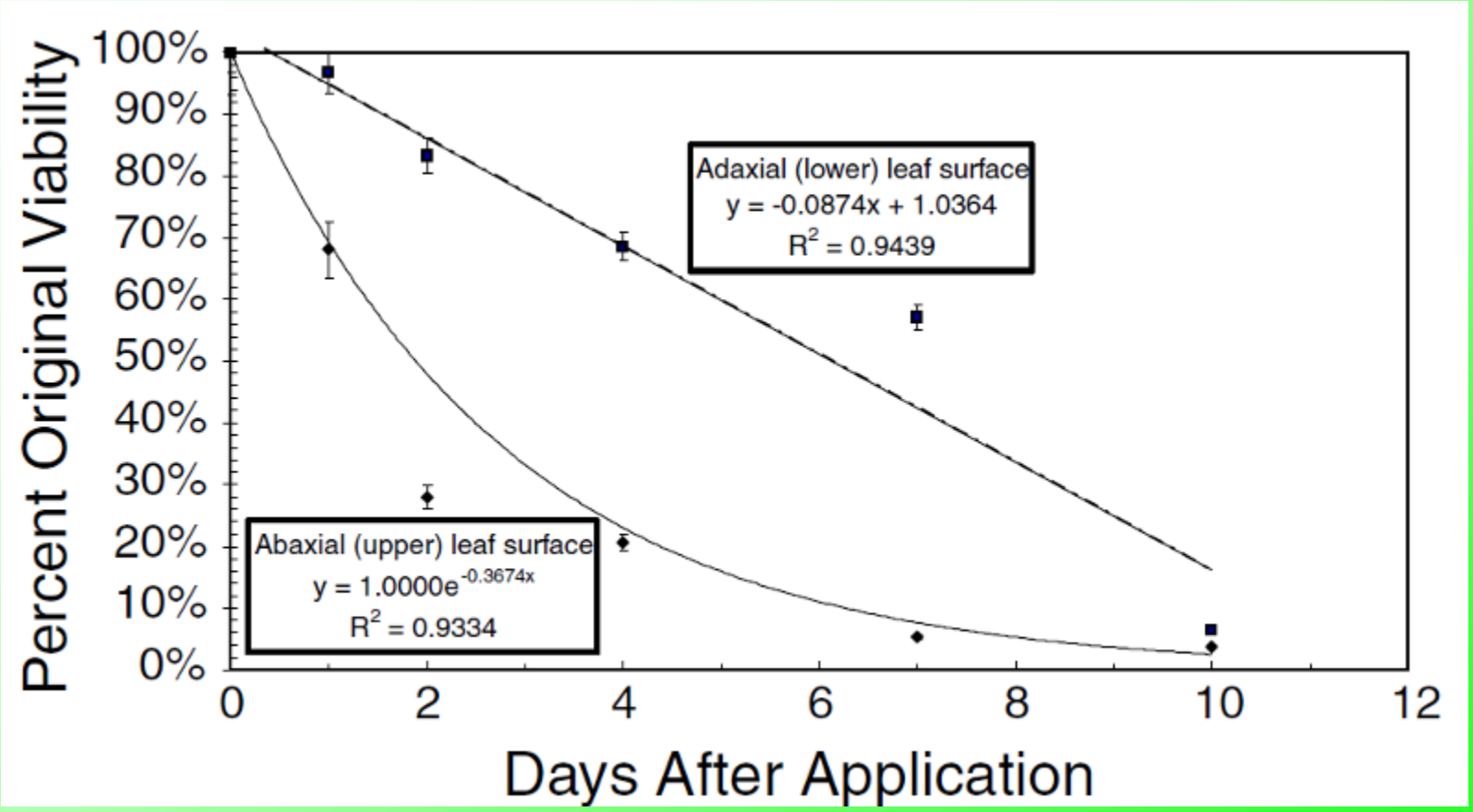


Fargues et al., 1996.
Mycopathologia 135:171-181

Caveat emptor

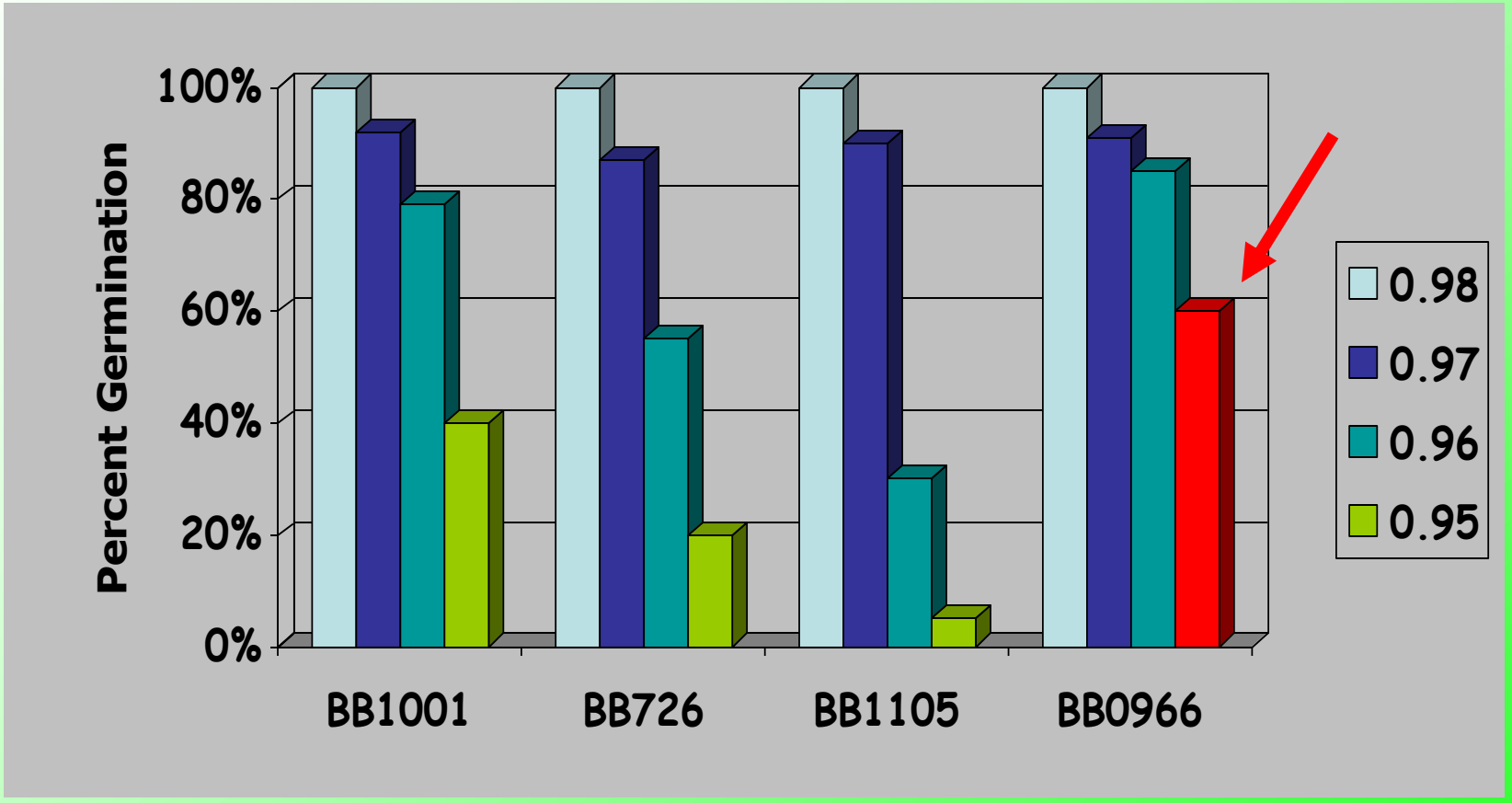
UV sensitivity testing in real world:

Propagule survival on shaded lower leaf surfaces can be longer than on upper surfaces (mimicked by lab tests)



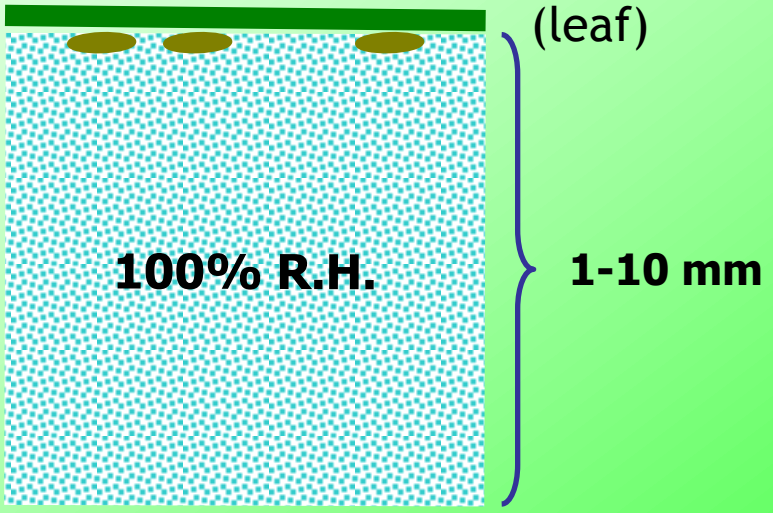
Tolerance to Low Water Activity

(spore germination)



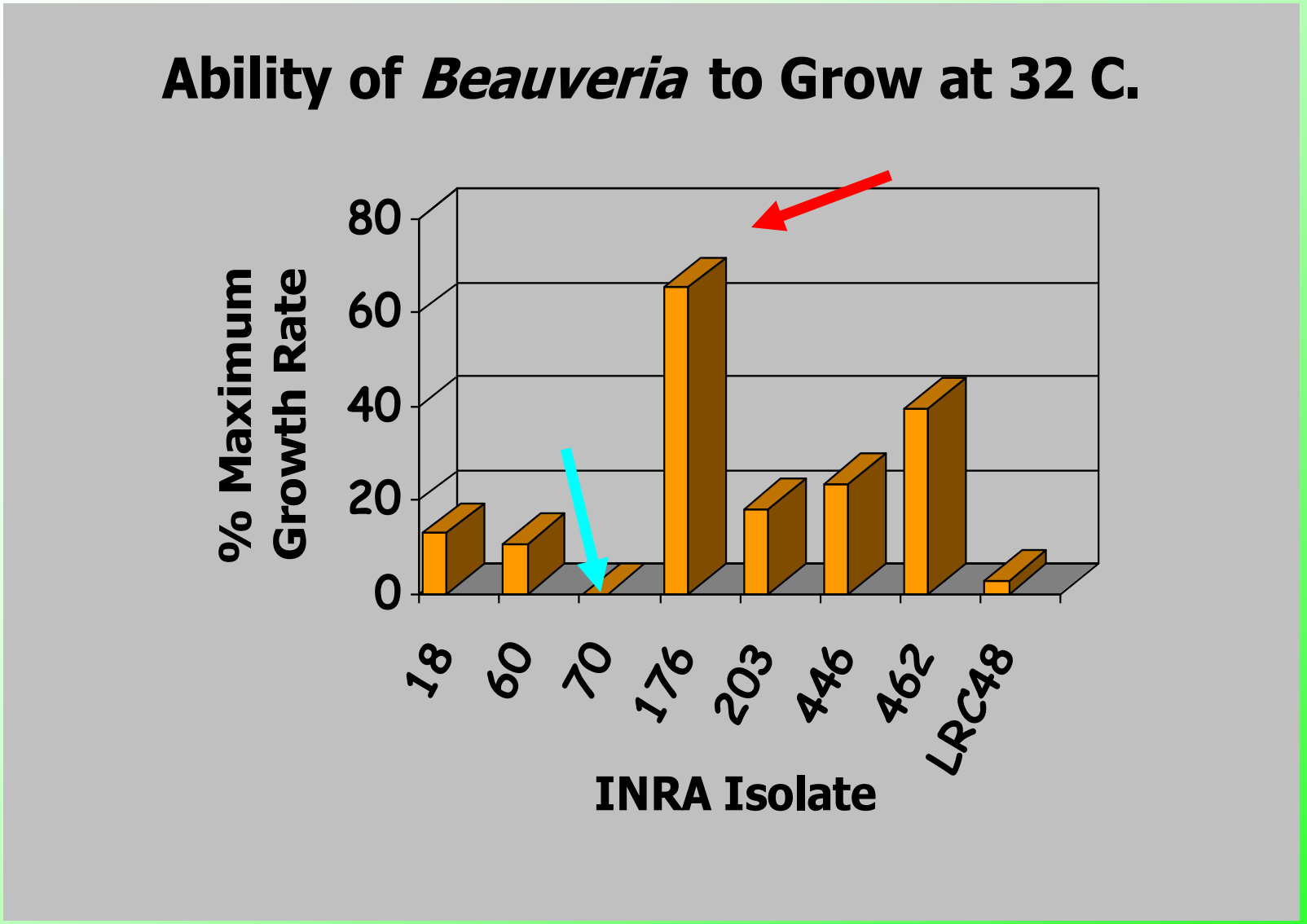
Caveat emptor

The Leaf Boundary Layer can greatly modify the phylloplane habitat R.H.



(Ambient: 50-70% R.H)

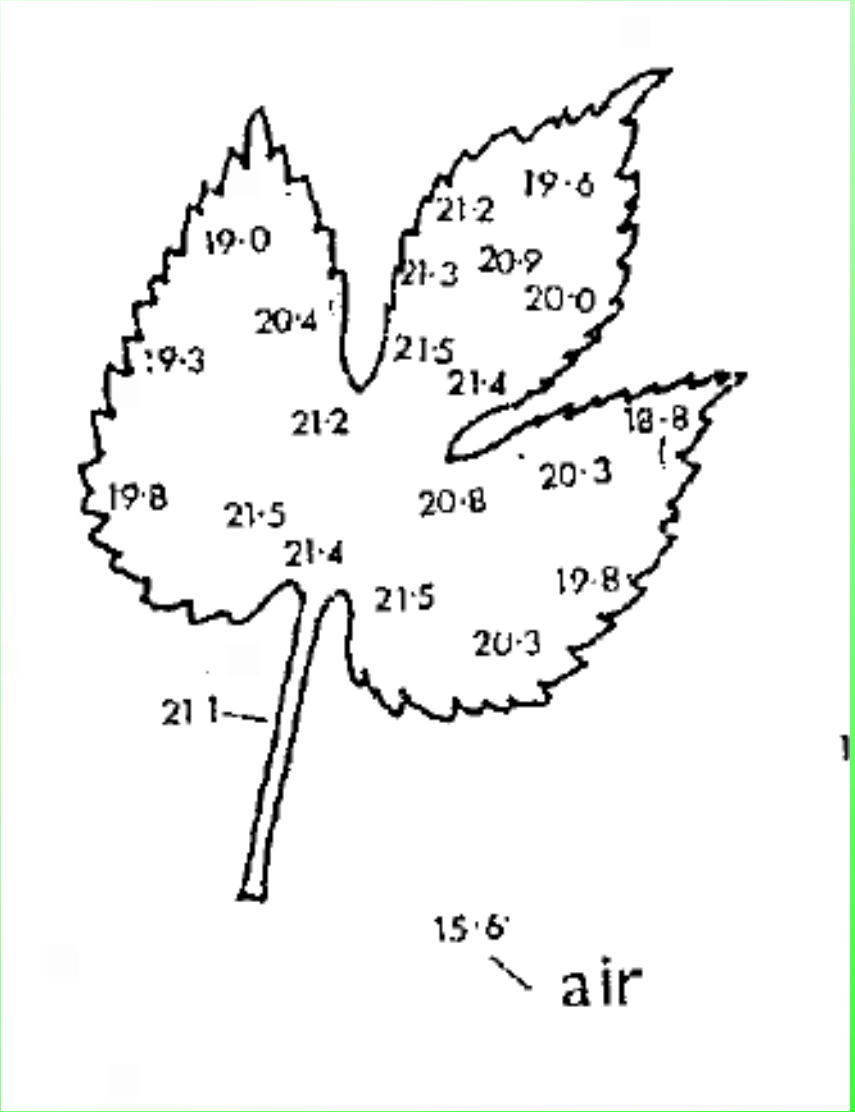
Tolerance to Temperature



Fargues et al., 1997. Mycologia 89:383-392.

Caveat emptor

The plant can modify leaf temperature



Burrage 1971

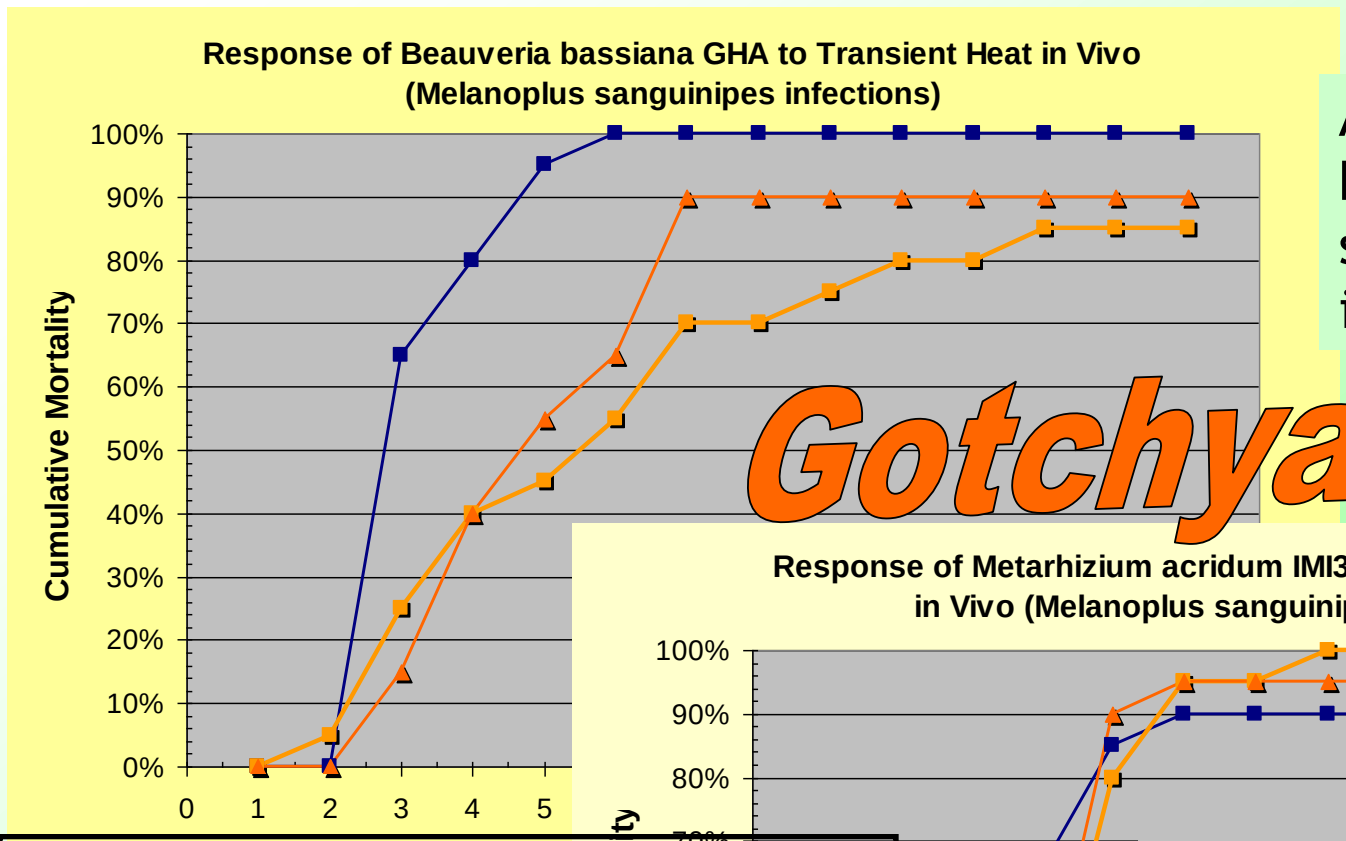
Caveat emptor

In vitro physiological responses to constant temperatures
not necessarily applicable in nature

For example,

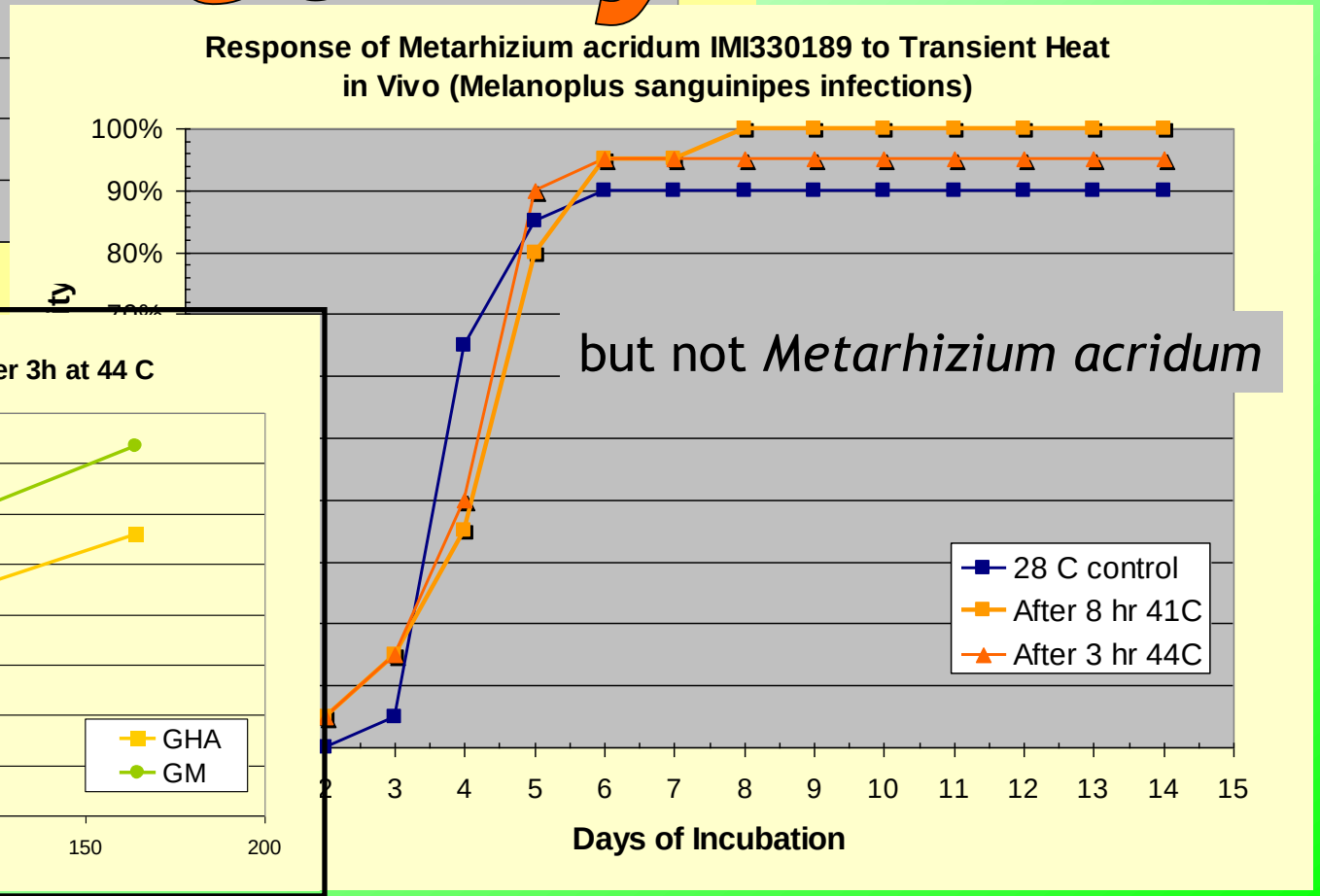
Orthopteran thermoregulation

and Overregulation
"Behavioral Fever"

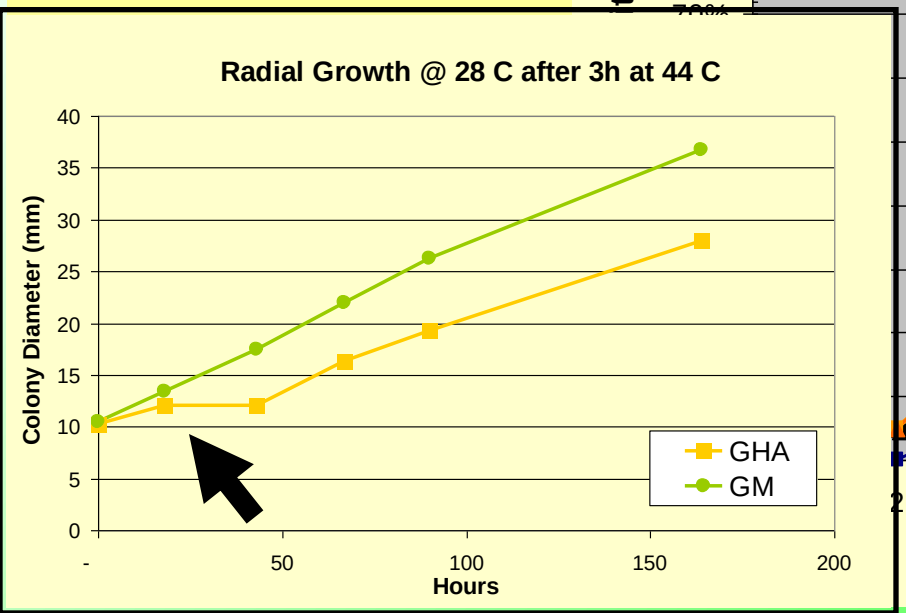


A <u>single</u> transient heat exposure will set back *Beauveria*, infection

Gotchya !



but not *Metarhizium acridum*



Caveat emptor

Some rhizosphere bacteria can inhibit germination of some EPF, at least in vitro

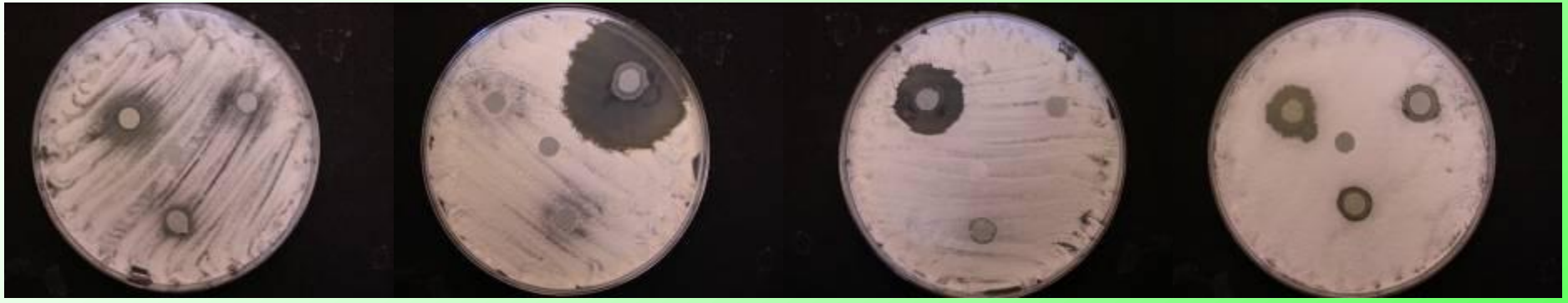
		Gram positive bacteria																	Gram negative bacteria														
		Micrococcaceae								*Bacillaceae*				*Microbacteriaceae*				Nocardiaceae	*Xanthomonadaceae*				*Xanthobacteraceae*	*Pseudomonadaceae*							*Enterobacteri*	
		Arthrobacter sp.	*Arthrobacter sp*	*Arthrobacter sp*	*Arthrobacter sp*	*Arthrobacter sp.*	*Arthrobacter sp*	*Arthrobacter viscosus*	*Micrococcus luteus*	*Bacillus macroides*	*Bacillus sp.*	*Bacillus mycoides*	*Bacillus mycoides BacJ*	*Microbacterium phyllosphaerae*	*Microbacterium liquefaciens*	*Microbacterium saperdae*	*Microbacterium lacticum*	*Rhodococcus sp.*	*Lysobacter sp.*	*Strenotrophomonas maltophilia*	*Stenotrophomonas maltophilia*	*Lysobacter antibioticus*	*Xanthobacter flavus*	*Pseudomonas sp*	*Pseudomonas sp*	*Pseudomonas fluorescens*	*Pseudomonas sp.*	*Pseudomas fluorescens*	*Pseudomonas sp.*	*Pseudomonas fluorescens*	*Yersinia enterocolitica*	*Yersinia sp.*
M. anisopliae	MA 1200	-	-	-	-	+	-	+	-	-	-	+	+	-	-	-	+	-	+	-	-	+	+	+	-	-	+	+	+	+	+	+
	F52	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-		-	-	-	-	+	+	+
	Canag	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	+	+	+
B. bassiana	GHA	-	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	+	-	+	-	+	+	+	+	+	+	+	+
	TM28	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	-	+	+	+
	TM86	-	-	-	-	-	-	-	-	-	-			-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	+	-

(Note inconsistency of patterns)

(Fuller, Jung, Jaronski in press)

Gotchya !

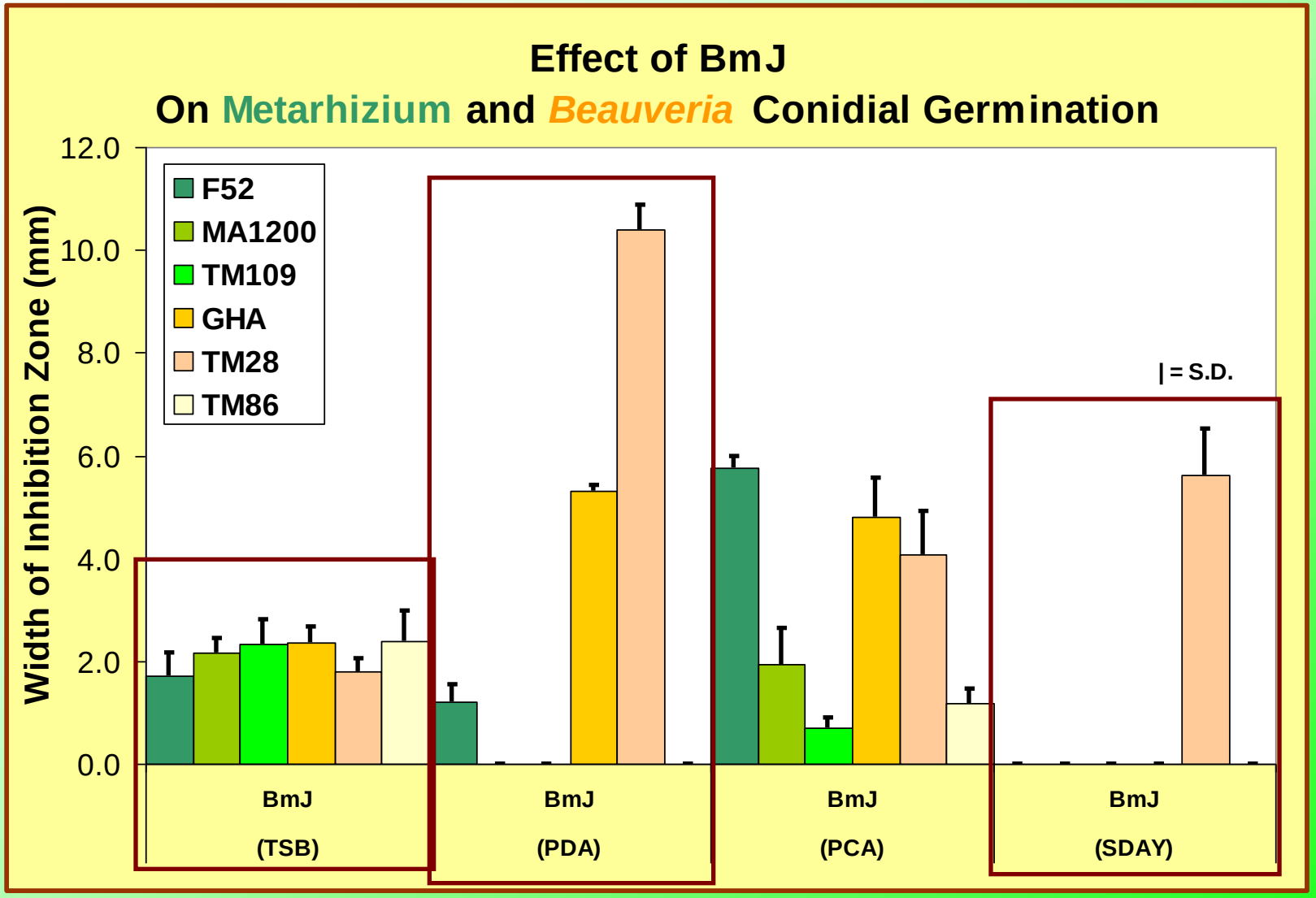
In studying mpca-microbe interactions,
The medium can affect the message



White growth: germinating Beauveria spores
Disks: same three bacteria on each medium

Gotchya !

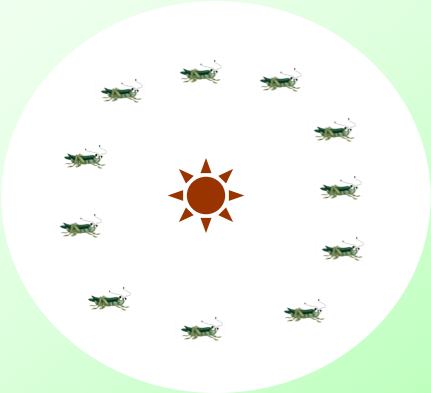
Medium (and fungus) can affect the message

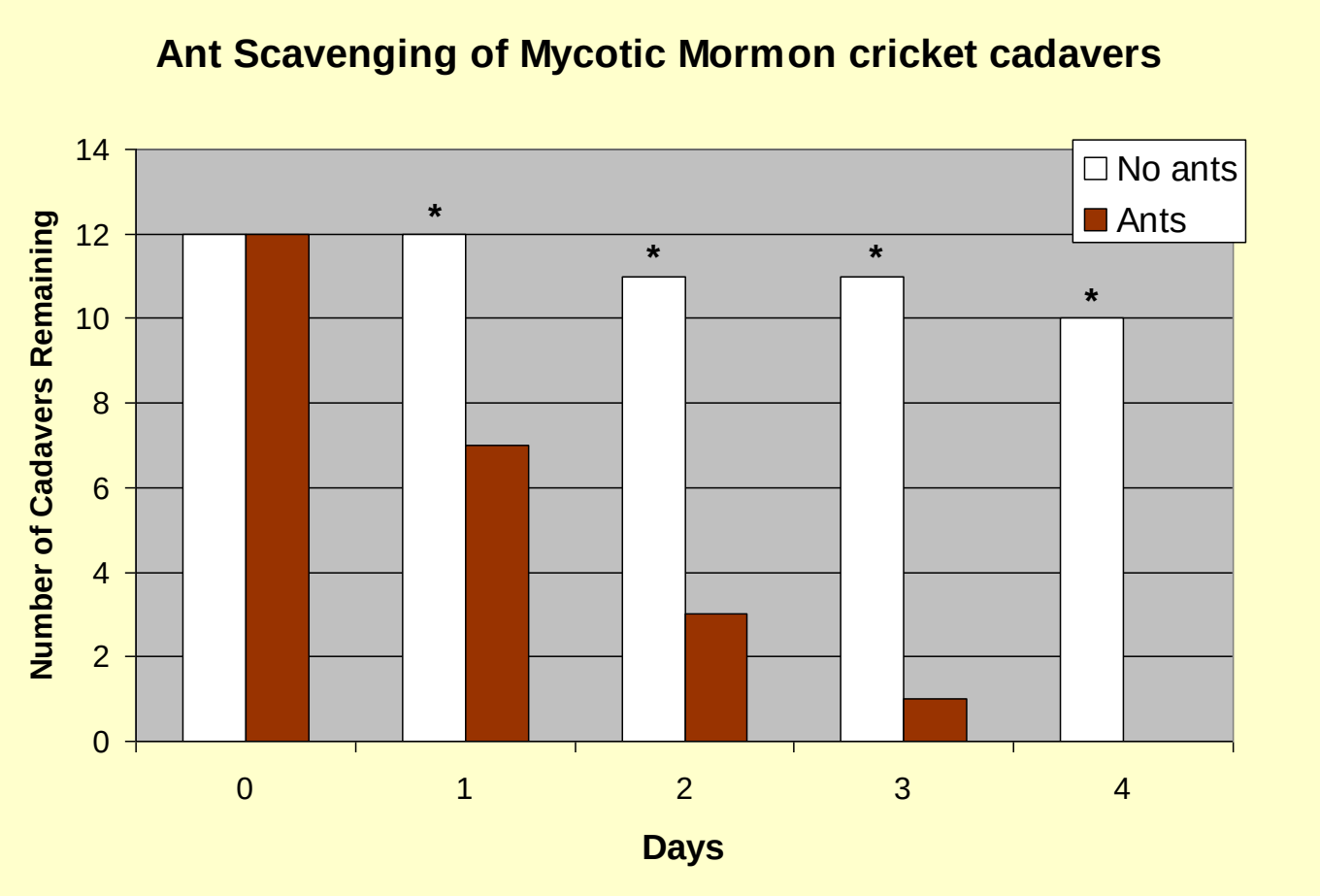


Caveat emptor

Scavengers can alter persistence

Ants are the major scavengers on the ground in many habitats
They will readily remove fungus-killed insects

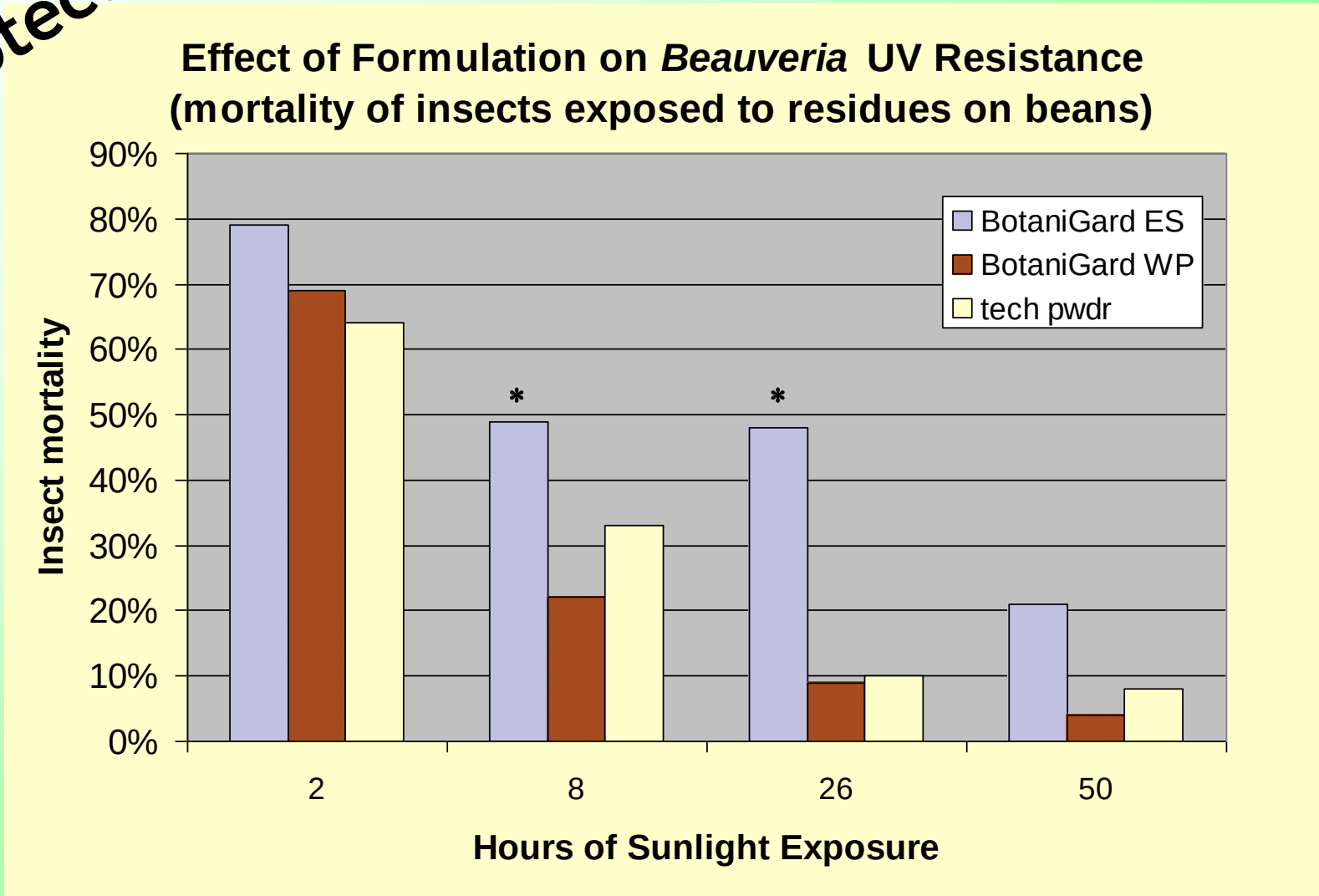




Human Manipulations can affect persistence

… via Formulations

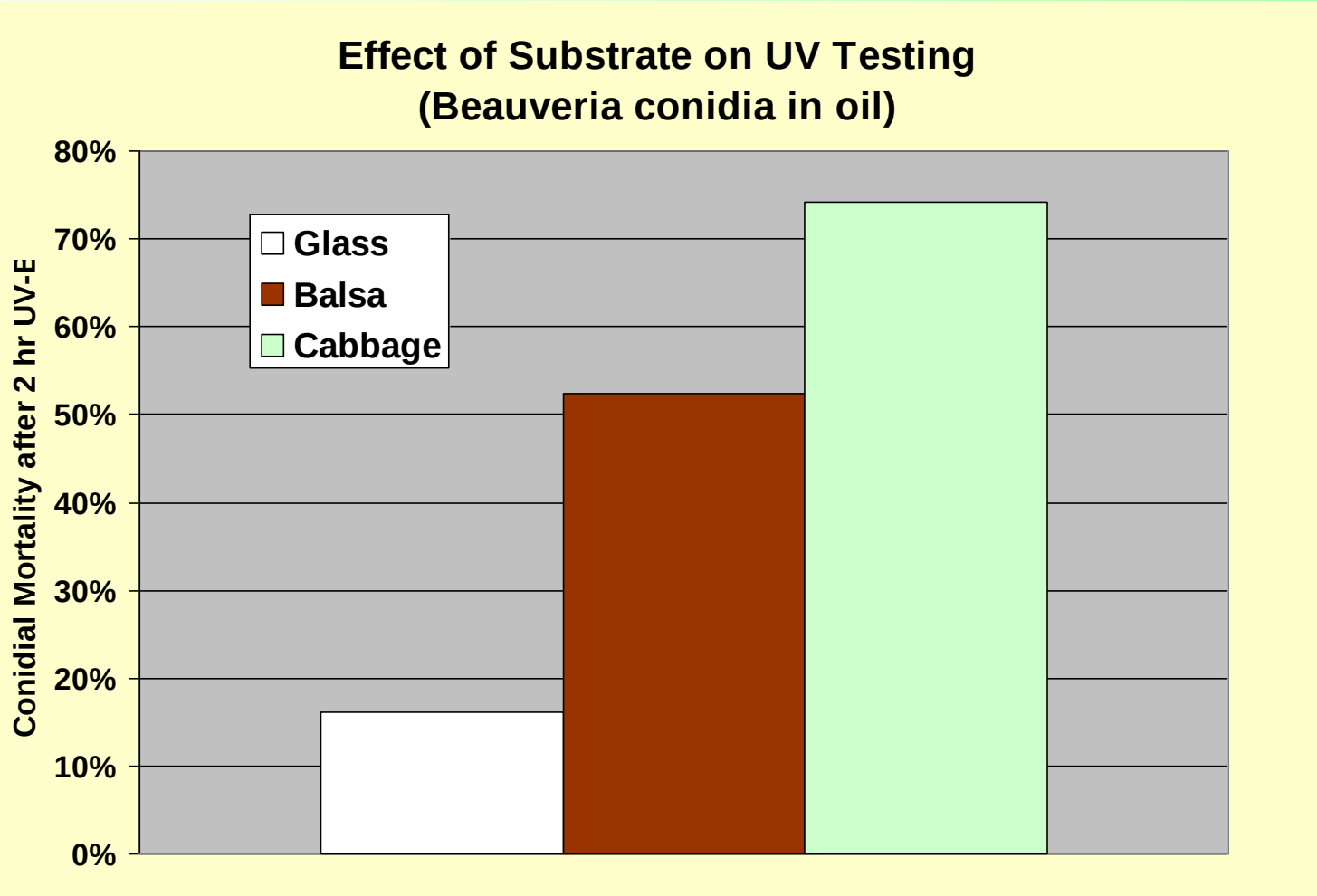
UV protection



Behle et al 2009 J. Econ. Entomol.

Gotchya !

But substrate in UV testing can affect results

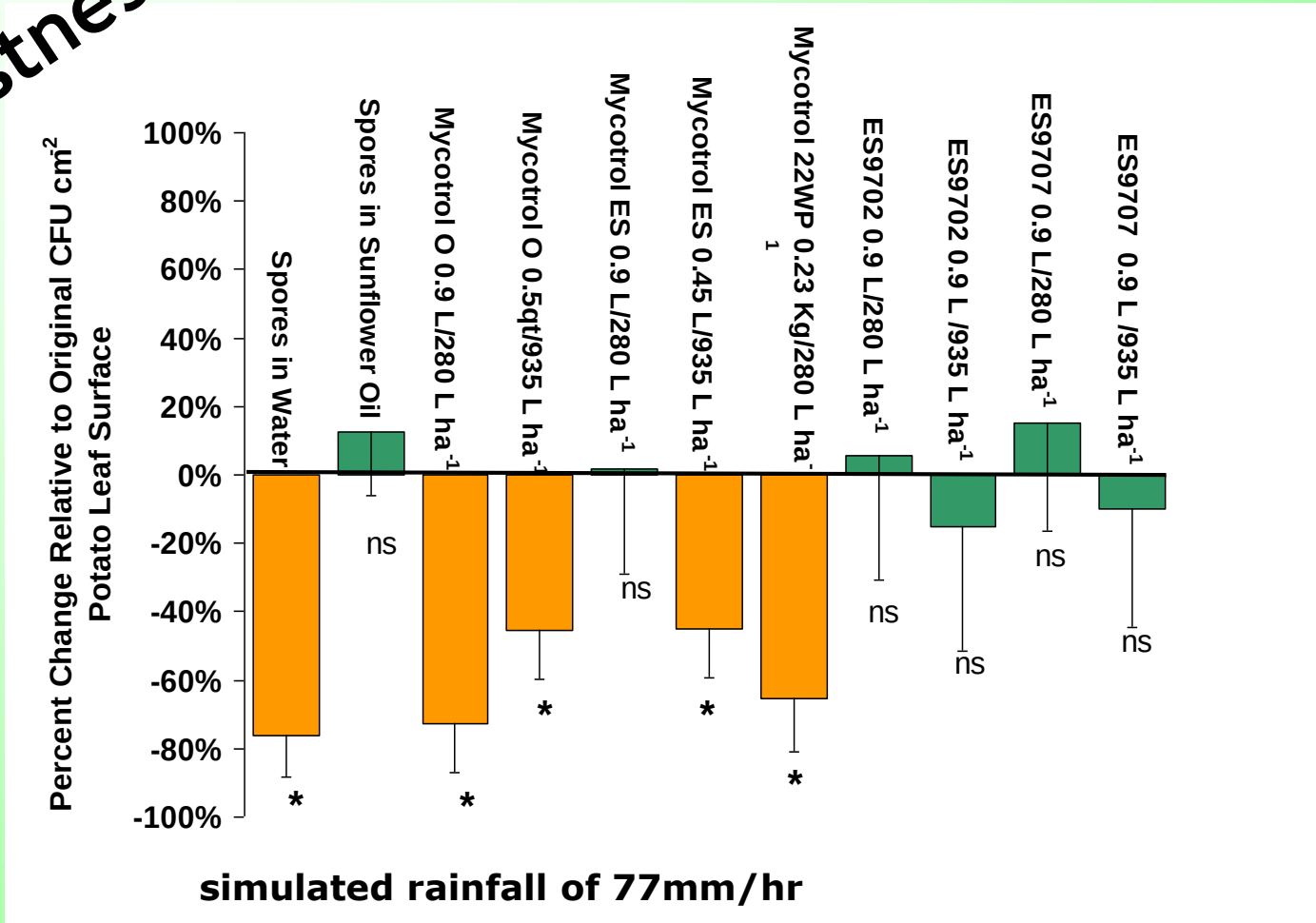


Behle et al 2009 J. Econ. Entomol.

Human manipulations can alter persistence

… via Formulations

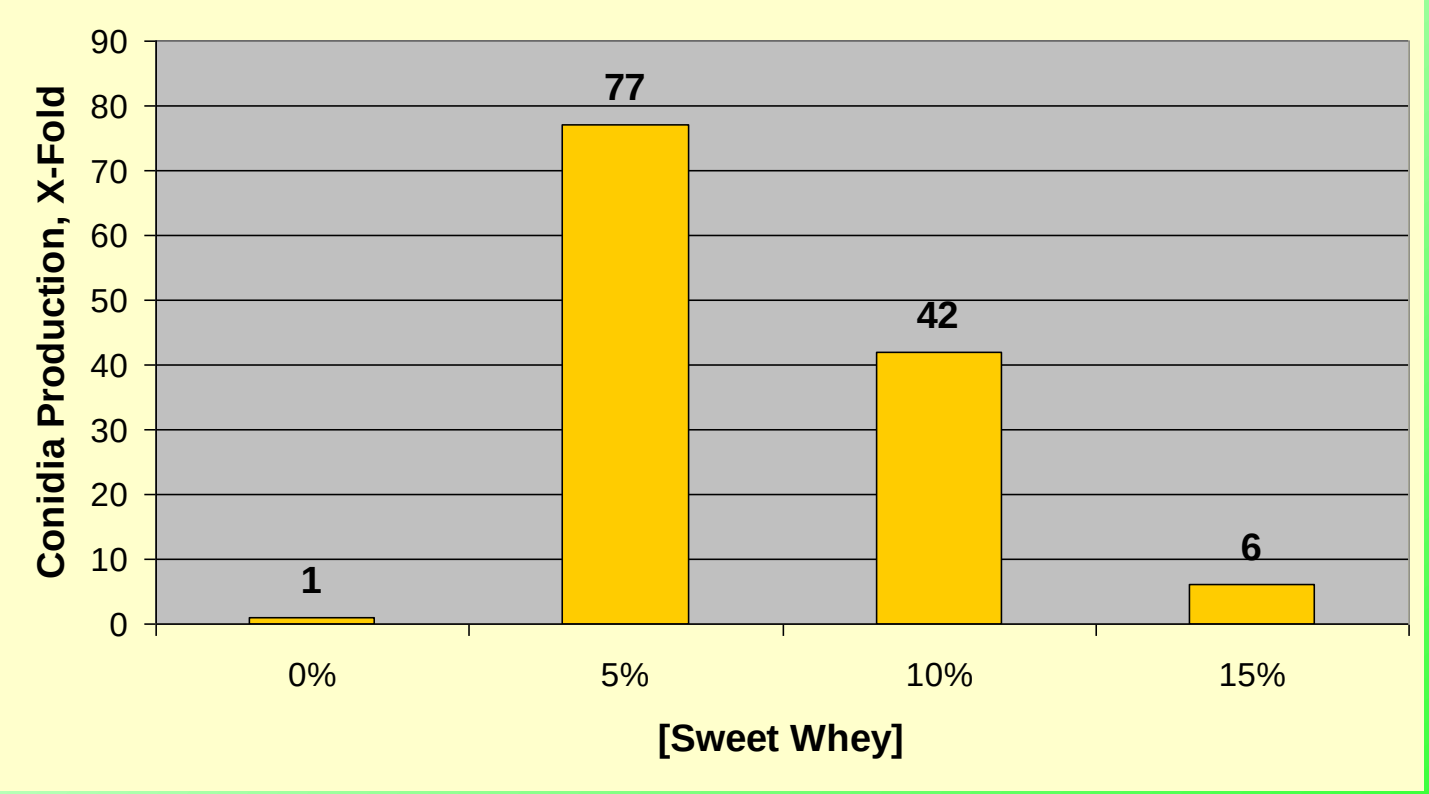
Rainfastness



Inglis et al. 2000 Biol Cont 18:55-64

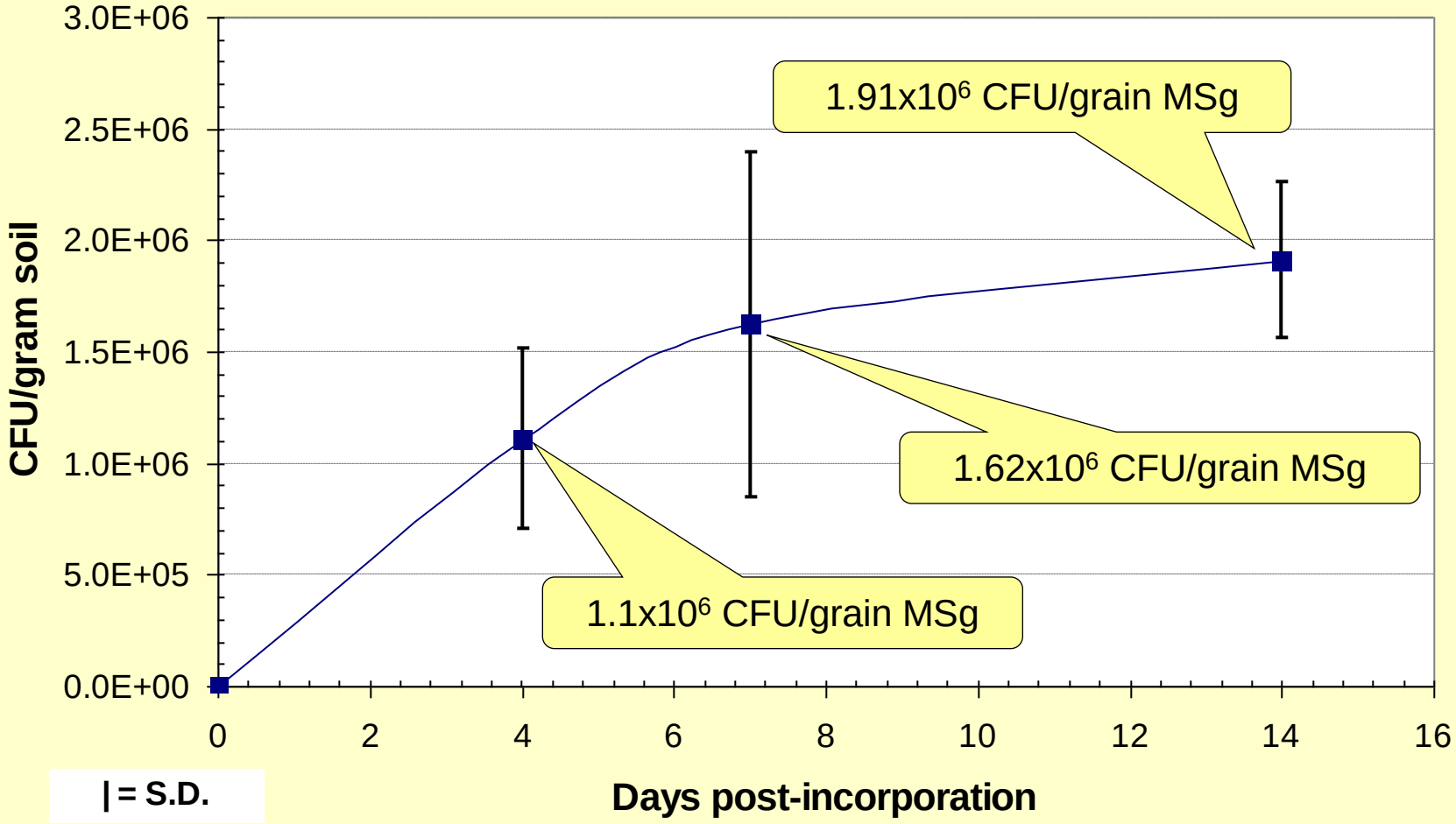
Human manipulations can alter persistence … via Formulations

***Lecanicillium* conidial production in sweet whey micro-factory**

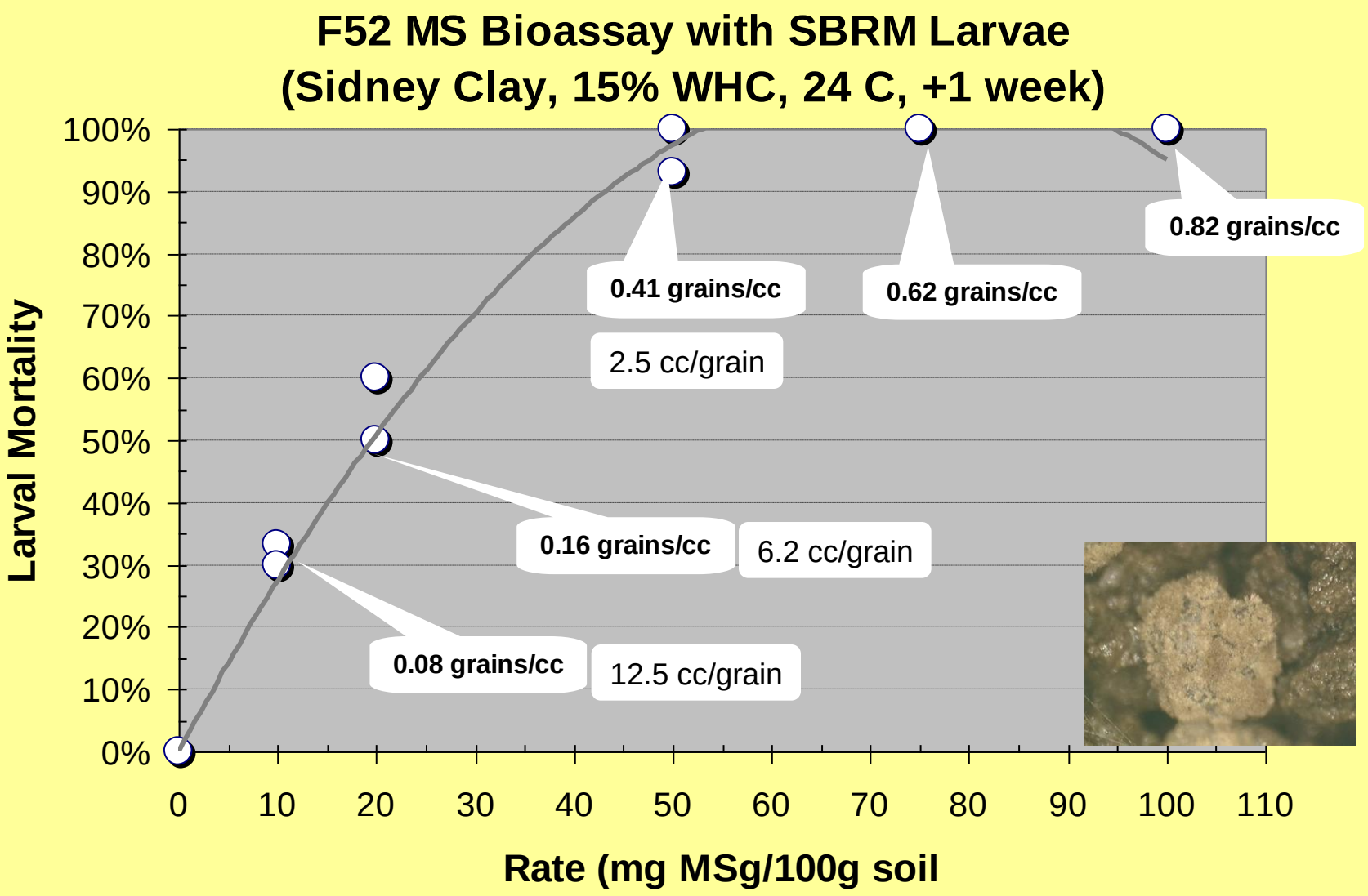


Costa et al 2008 WIPO Pat Appl WO2008_073843

CFU Generation by Microsclerotial Granules in Clay Soil
(@ 1 mg MSg / gram soil; 15% WHC = -2.33 MPa; 24 ^{o} C.)

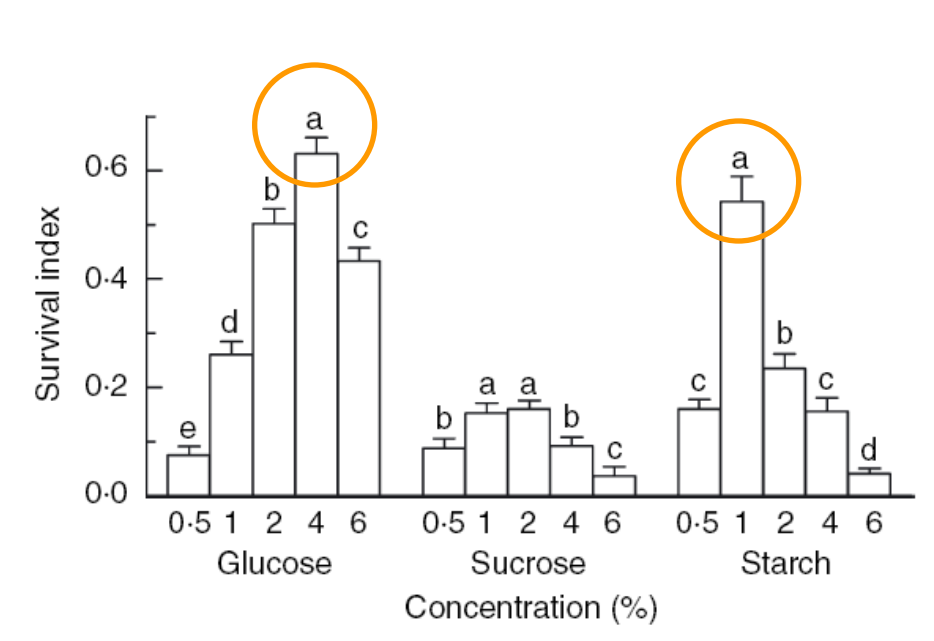


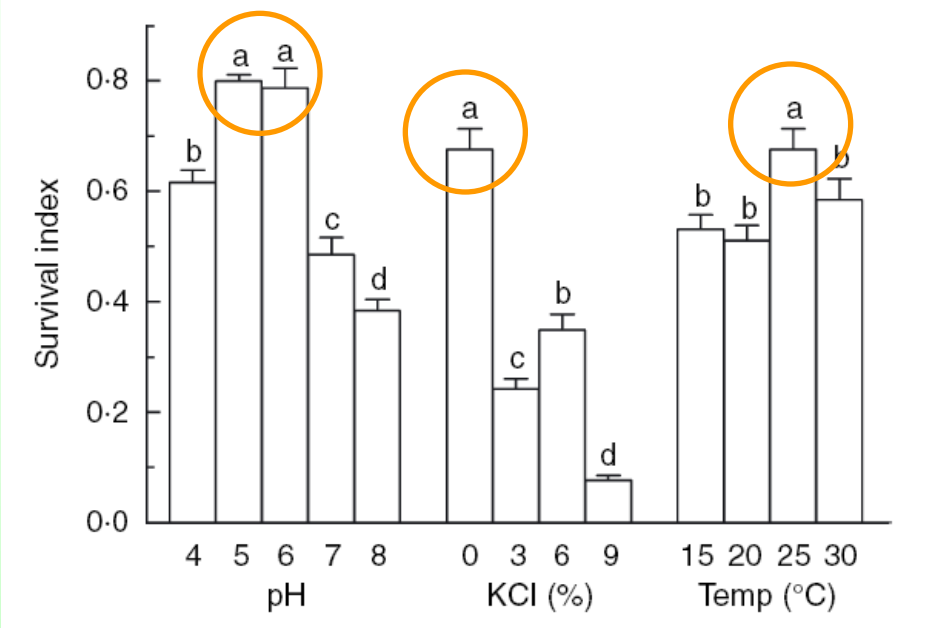
Jaronski and Jackson 2009. Bull. IOBC wprs 45: 275-278



Jaronski and Jackson 2009. Bull. IOBC wprs 45: 275-278

Human manipulations can alter persistence … via Fermentation





Fermentation conditions can affect thermotolerance of EPF conidia

Ying and Feng. Letters in Applied Microbiology 43 (2006) 331–335

Human manipulations can alter persistence
… via Fermentation

Stressful growth medium (a_w = 0.995):

— Caused ↑ [erythritol] in EPF conidia

— Accelerating germination over range of aw

— *Beauveria, Metarhizium, Lecanicillium, Isaria*

— faster infection (and pathogenesis) of aphids

But effect varied by species

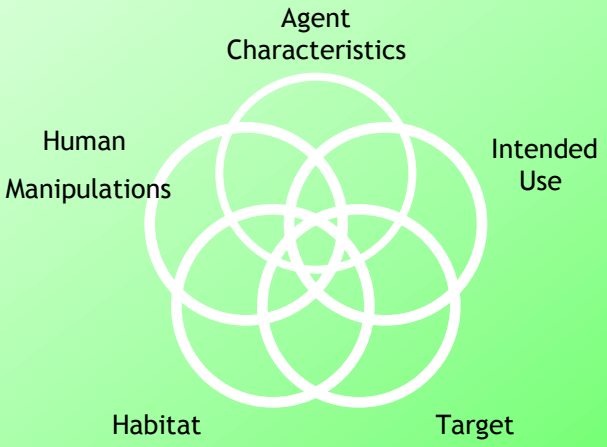
Andersen et al. 2006 Environmental Microbiology

How can we predict persistence? … realistically

- generalizations from the pathogen group ?
- laboratory studies *in vitro, in vivo*
- *In silico* models
- outdoor “lab” studies
- field application and monitoring ?

(*“Where the rubber meets the road”)*

Consideration of persistence in assessing the risk of a microbial agent:



- Needs to consider
  - → the agent,
  - → its use pattern
  - → its habitat
  - → possible human manipulations
- Situation is complex; few good generalizations

Persistence has to be evaluated

→ case by case basis ?

→ under as realistic field conditions as possible.

Thank you for your attention

(St. Urho – patron saint of grasshopper control - drove the grasshoppers out of Finland)