MYCOINSECTICIDES 101

Stefan T. Jaronski, Ph.D.

USDA Retired,

Adjunct Professor, Virginia Polytechnic and State University

Jaronski Mycological Consulting LLC, Blacksburg VA

MEET THE CURRENT PLAYERS,

Beauveria

Metarhizium

Cordyceps, the fungus formerly known as *Isaria*

Akanthomyces, aka *Lecanicillium*, aka *Verticillium*

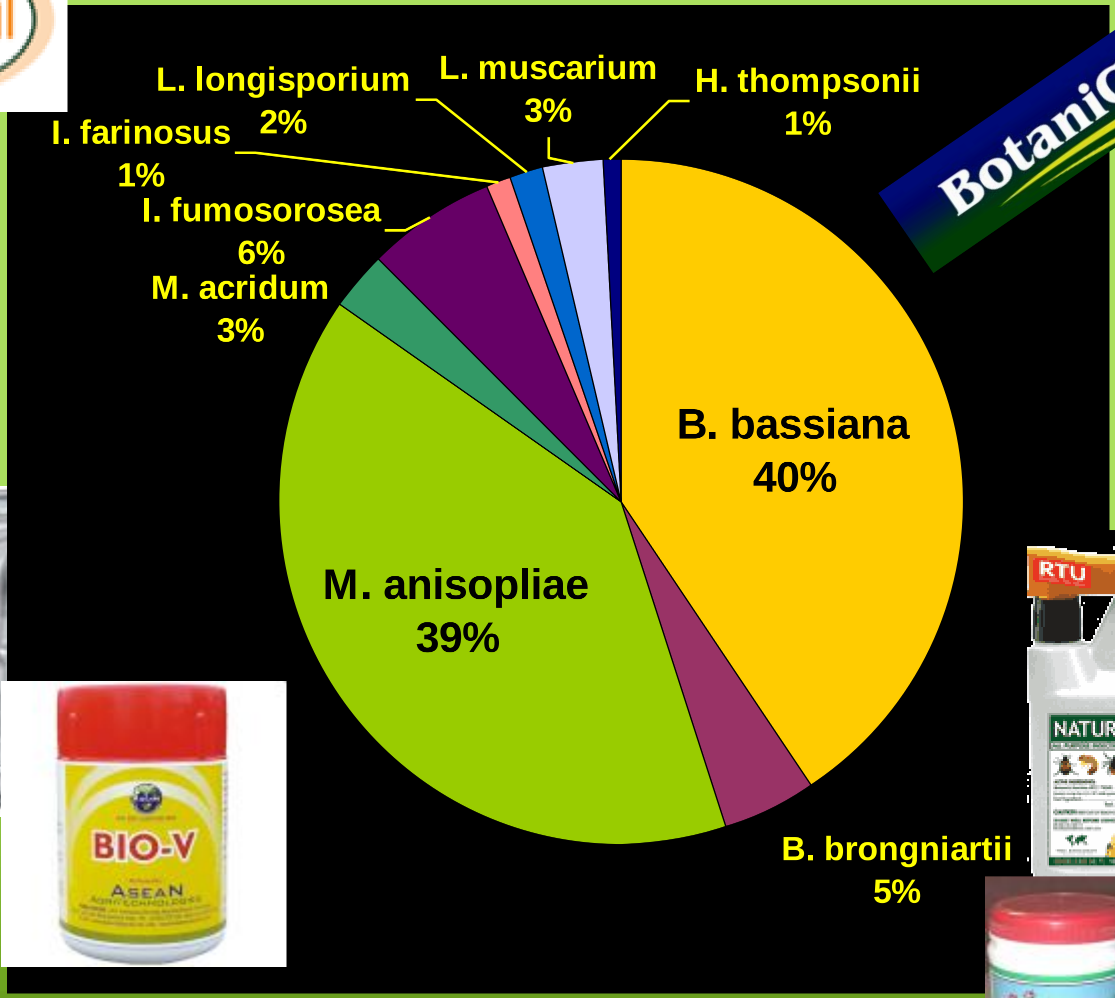
Using fungi to manage insect pests is not a new idea

- **1880s** Metchnikoff and Krassilichik in Russia

- **1880s-1890s** Luggar and Snow, in Kansas and Midwest

"Maybe we can actually control insect pests with microbes."

Mycoinsecticides:
110 active, commercial products in 2006
200+ worldwide 2020



Faria and Wraight Biological Control 43 (2007) 237–256
Mascarin and Jaronski World J. Microbiol. Biotechnol. (2016) 32:177

Mycoinsecticides: Currently registered in the US, Canada

Beauveria bassiana

- **GHA:** BotaniGard, Mycotrol (Certis USA);
  Aprehend (ConidioTec)
- **ANT-03**: BioCeres (Anatis Bioprotection)
- **PPRI 5339**: Velifer (BASF)
- **R444:** Bassidor / Bb-ProTec (Andermatt)
- **CFL-A:** Fraxiprotec (Canada, CDG Environment)
- **BW149:** Principle WP (BioWorks, in process at EPA)
- **ATCC 74040**: Naturalis (Lallemand Plant Care)
- **HF23:** balEnce (JABB of the Carolinas)

Metarhizium brunneum (anisopliae)

- **F52:** LalGard M52 (Lallemand Plant Care)

Cordyceps (Isaria) fumosorosea

- Apopka 97: PFR97, Preferal (CertisBio USA)
- FE 9901: Isarid (Futureco Bioscience, Koppert)

Host Specificity e.g. *Beauveria*

1. *Beauveria bassiana* as a species attacks all insects, many spiders, some ticks, mites
2. *Beauveria* strains have different *relative* specificities

	Efficacy			
	BB1	BB2	BB3	BB4
Whitefly	1	15	60	1000
Aphids	2	10	40	1000
Lygus	4	1	20	1000
Beetles	7	40	10	1000
Grasshoppers	10	9	50	1000
Armyworm	20	100	1	1000
Fly Maggots	100	200	50	1000
Honeybees	1000	500	500	1000
Spider MItes	500	500	500	1

The lower the number, the more effective it is

Every strain is different: a "real world" example
Relative *Beauveria* strain efficacy (lab assay) for 5 lepidoptera

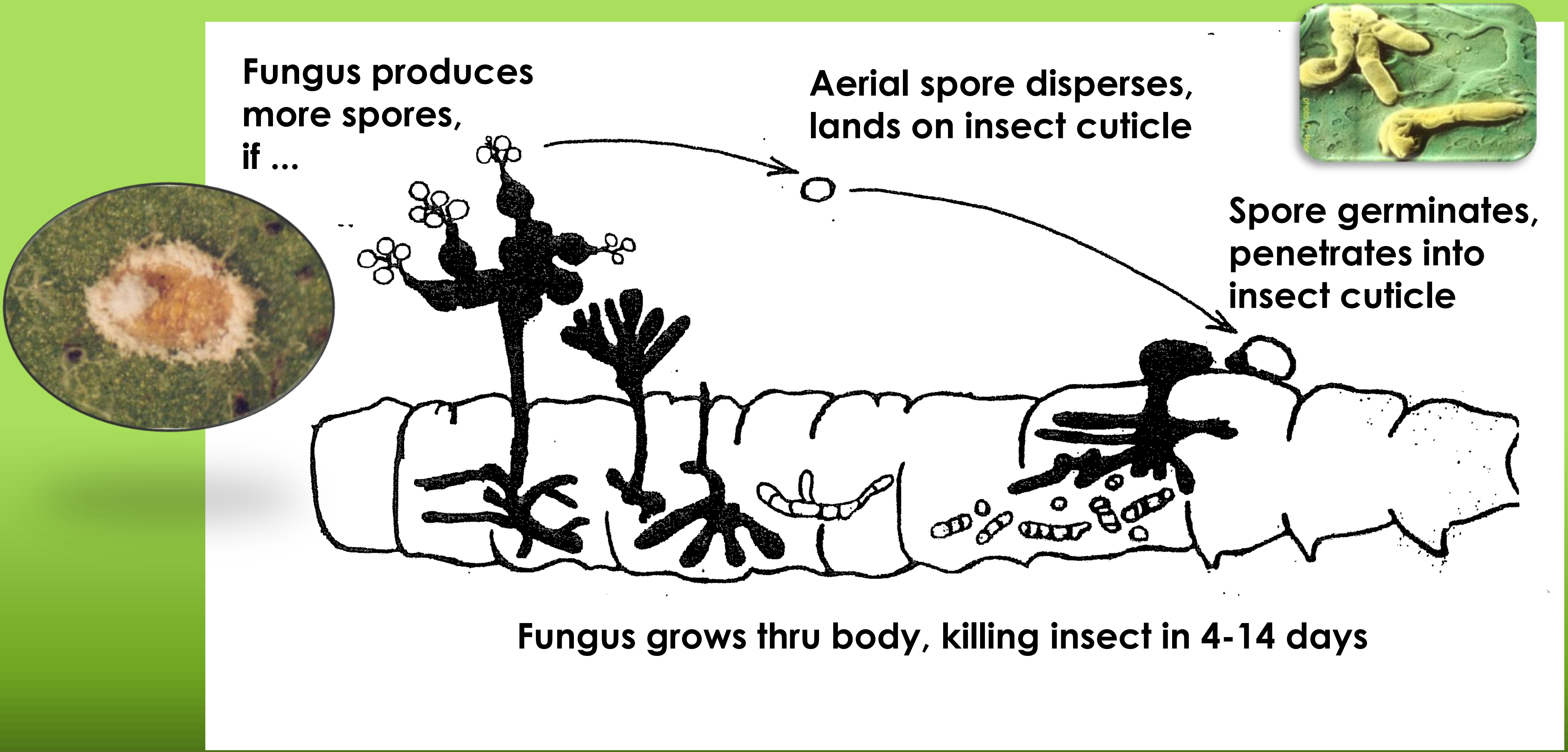
Fungus	Diamondback Moth	European Corn Borer	Corn Earworm	Fall Armyworm	Beet Armyworm
1060	37	1	43	22	14
1200	1	18	3	1	1
1513	30	5	41	5	12
1966	2	20	28	27	17
1023	28	2	40	34	3

Wraight et al 2010

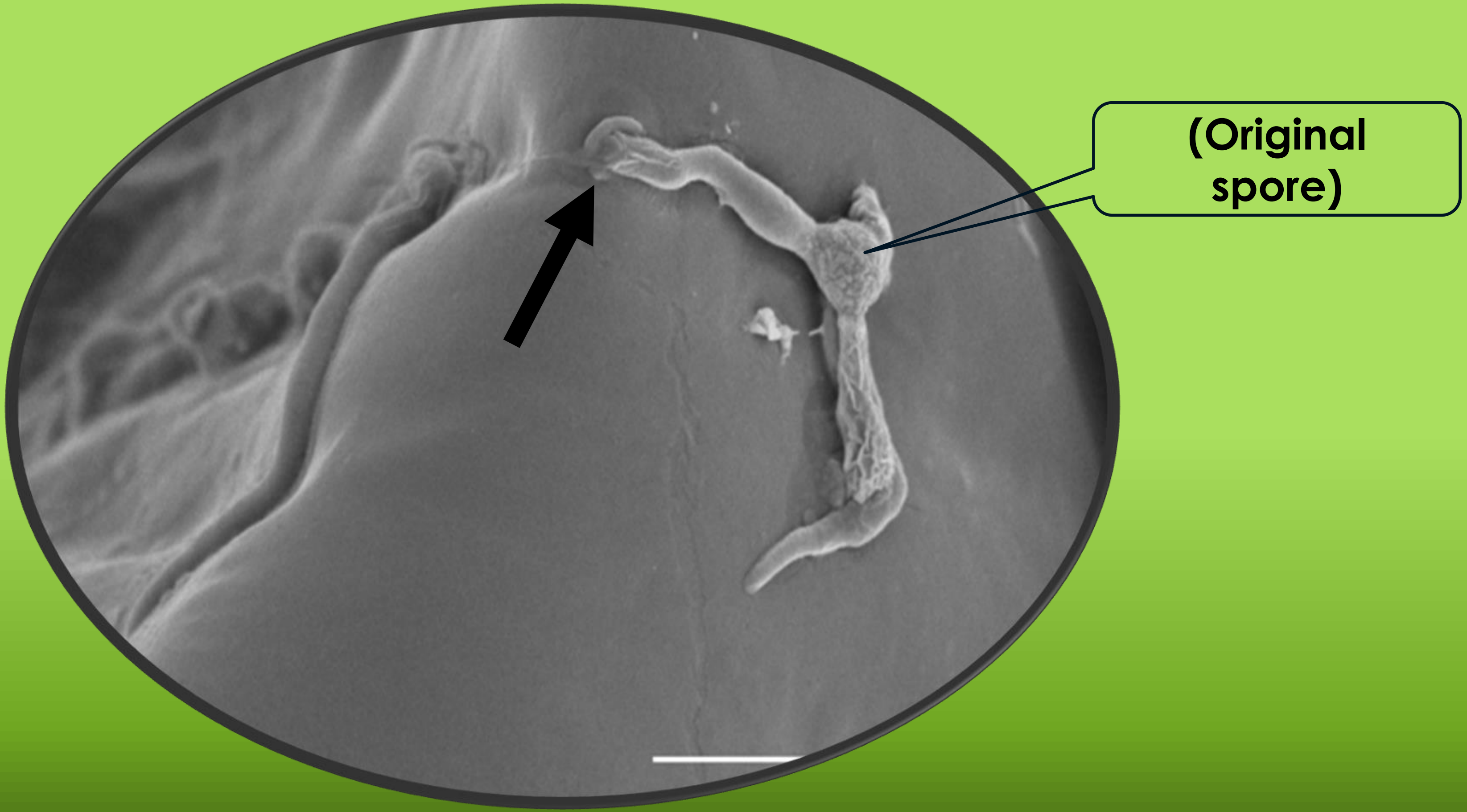
Number is its rank out of **43** strains tested

How do these fungi work?

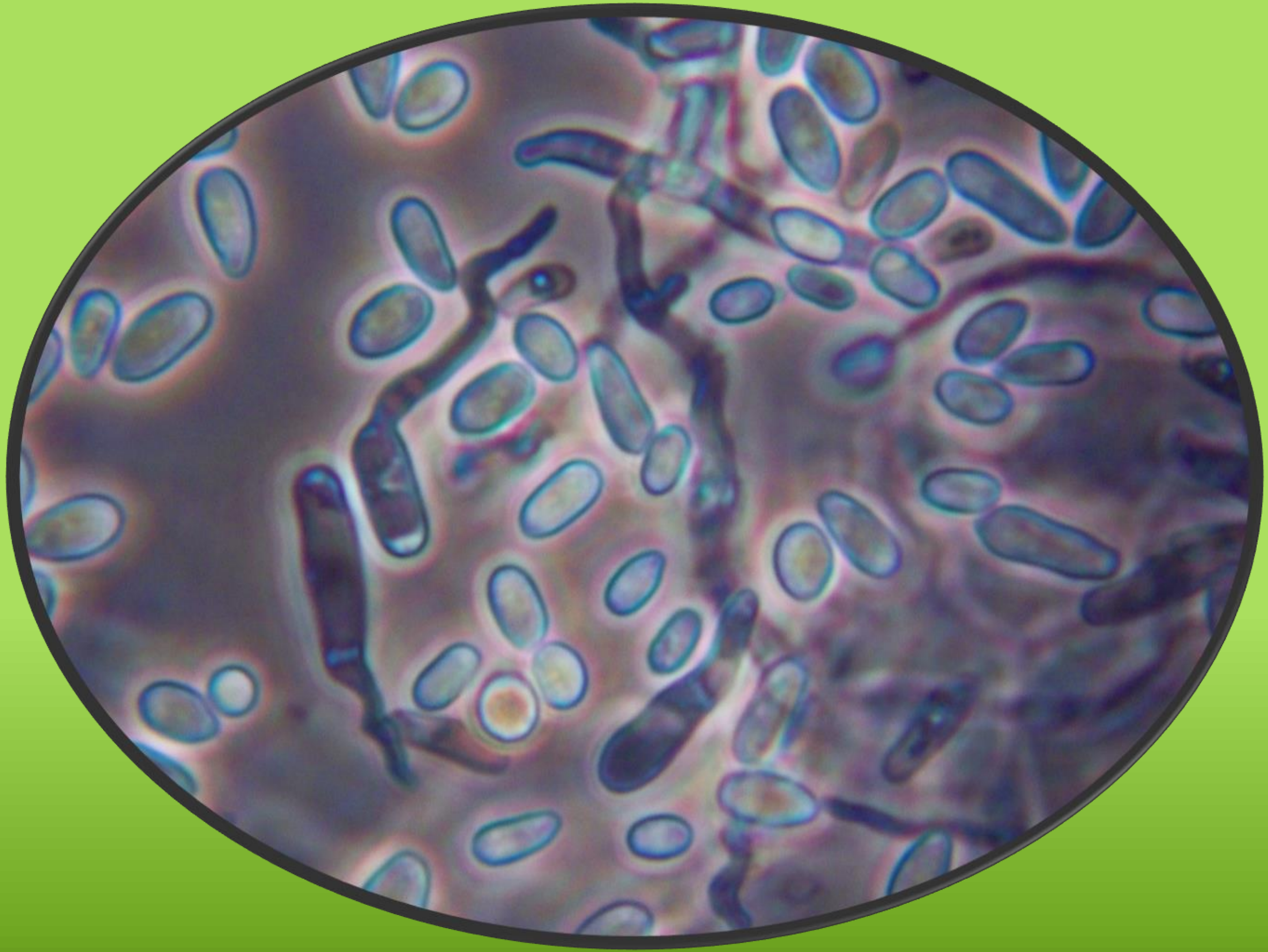
(Think "fatal athlete's foot (or body)" of insects)
i.e. CONTACT agents



Beauveria spore germinating on insect's cuticle – note how it grows out, seeks a good penetration point and goes into the cuticle (arrow)



Fungus multiplying inside the haemolymph, the blood, of an insect



So how do these fungi kill their hosts?

- **Metabolites…**
  to defeat insect's defenses
  to disrupt metabolism
  to combat saprophytic microbes on insect's death
- **Enzymes…**
  to "eat" nutrients inside the insect, lipids, carbohydrates, amino acids

Infected insect dies within 3-14 days depending on its size, dose received

Progression of fungus emergence, sporulation

Day 0

Day 3

Day 4

BUT outgrowth and sporulation requires <u>unbroken, ~100%</u> humidity for 2-3 days

in most cases fungus dies with insect

Day 6

Mammalian, human, safety

These fungi -- Beauveria, Metarhizium, Cordyceps, Akanthomyces -

are SAFE

They do not infect humans
(except in rare instances, with severely immunocomprised people).

NO “The Last of Us”
with these fungi

There is some sensitization, allergic potential, esp. with wettable powder formulations.

May cause sensitization. May cause eye irritation.

> Avoid contact with eyes, skin and clothing.
> Avoid inhaling/breathing mists.
> Wear waterproof gloves,
> a long sleeved shirt and long pants, shoes plus socks
> a dust-mist filtering respirator/mask (NIOSH TC-21C, N-95, P-95, R-95 or HE filter for biological products), when handling, mixing/loading or applying the product and during all clean-up and repair activities.

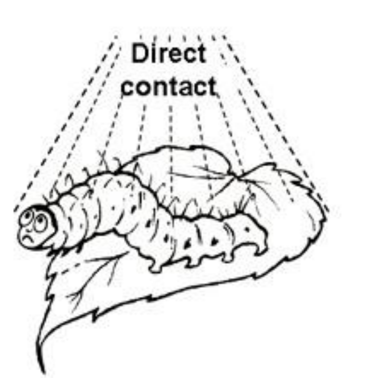
Restricted Entry Interval-
4 hours ☺

Read the product label and SDS!
Follow the product label and SDS!
Always!

A fact of life and death

One needs a LOT of spores

Numbers, numbers, numbers



- ***A single spore does not make a lethal infection***
- LC_{90} Beauveria strain GHA (lab bioassay)

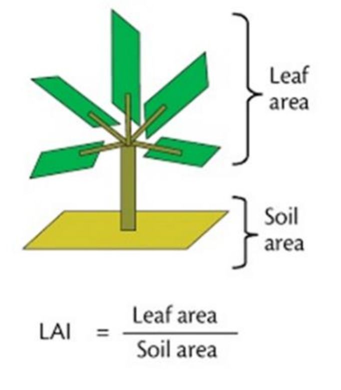
  100 spores/mm² sprayed surface (Whitefly)

  28 spores/mm² sprayed surface (Diamondback Moth)

A flat acre = 4,050,000,000 mm² ("4 billion")

But, allowing for area of foliage

32,400,000,000 mm² in 1 Ac canopy w/ "L.A.I." of 8



= **$9.1x10^{11}$ spores** (DBM), **$3.24x10^{12}$ spores** (WF) **/ Ac**

= **0.09 qt BotaniGard ES/Ac** **0.3 qt BotaniGard ES/Ac**

BUT that's in a perfect world …

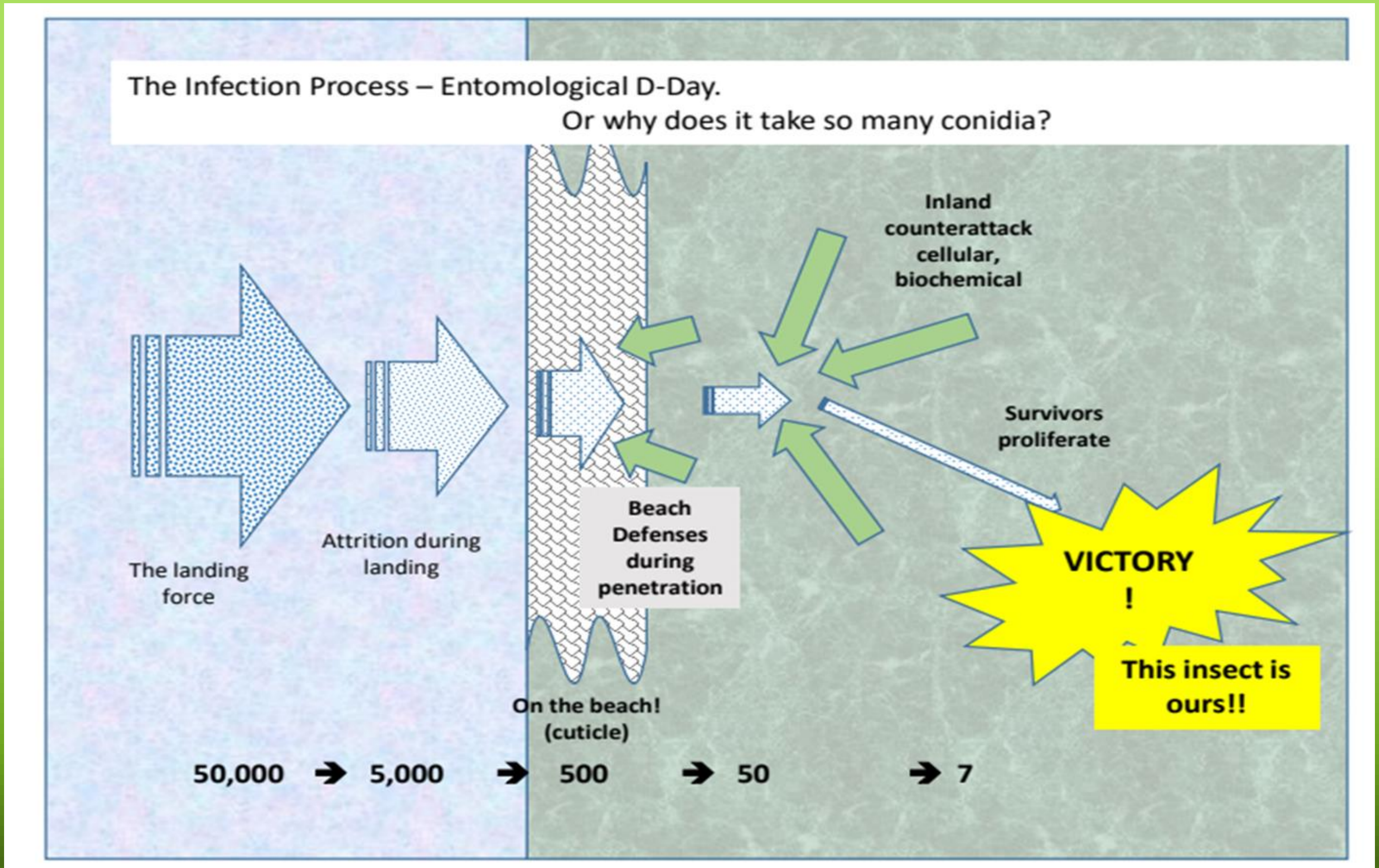
A fact of life and death

One needs a LOT of spores to kill an insect

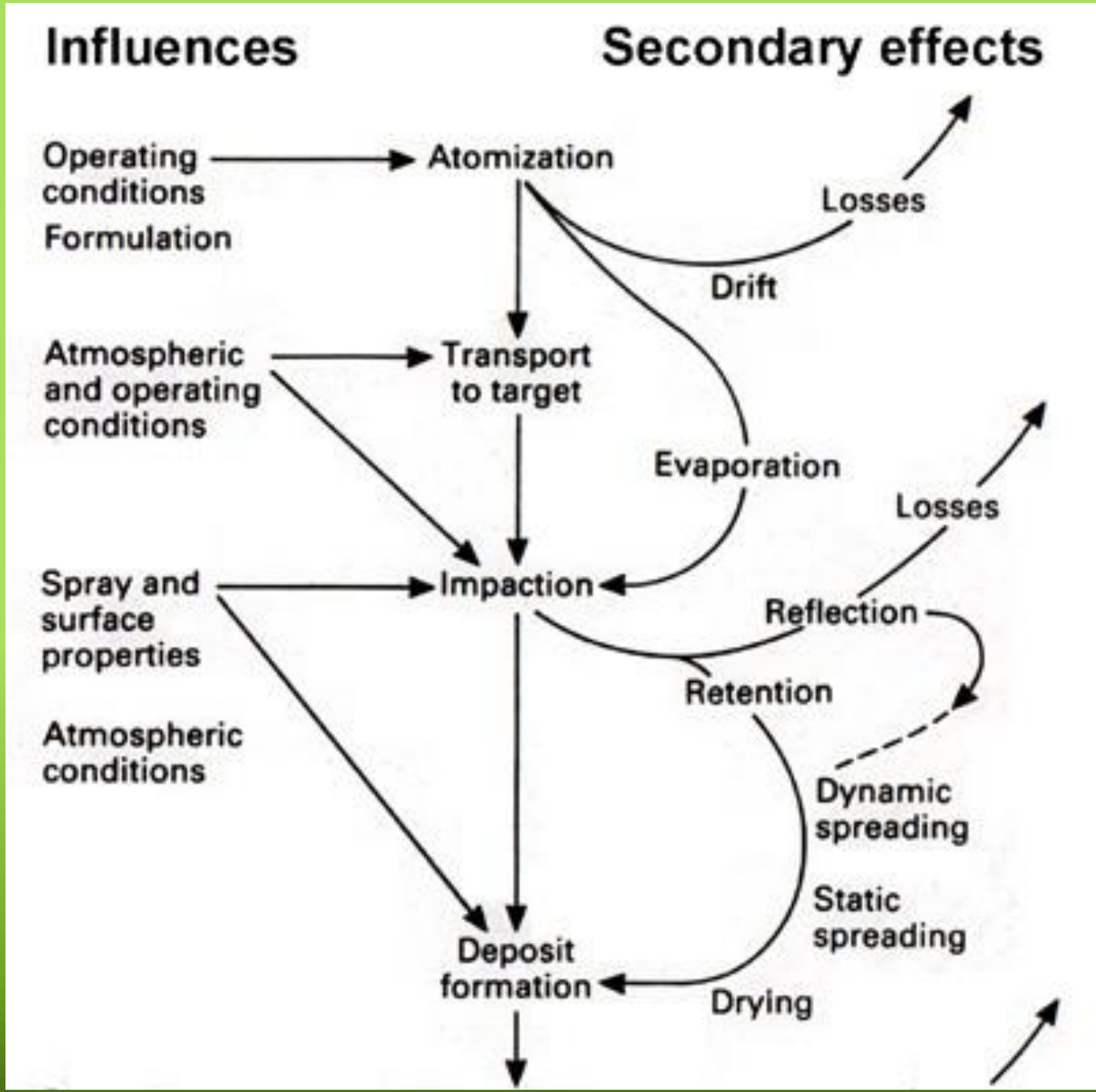
WHY?

Numbers, numbers, numbers

(an LD95 can be 100s, 1000s of spores)



Delivery of fungus spores is inefficient
often VERY INEFFICIENT



Therefore,

DBM: 0.1 qt ➔ 1 qt/Ac

WF: 0.3 qt ➔ 2-3 qt/Ac

Per spray

So: Coverage, coverage, coverage!!! Aim for the insects, or where the insects will be !!

➔ Maximize spores / area of leaf surface, where the insects are

- **Fine spray**
- **Good wetting agent (organosilicone ?)**
- **Do not spray to runoff, but stop before runoff so leaves are just wet, let the wetting agent work.**
- **CRITICAL with Whiteflies to get spray to leaf undersides.**

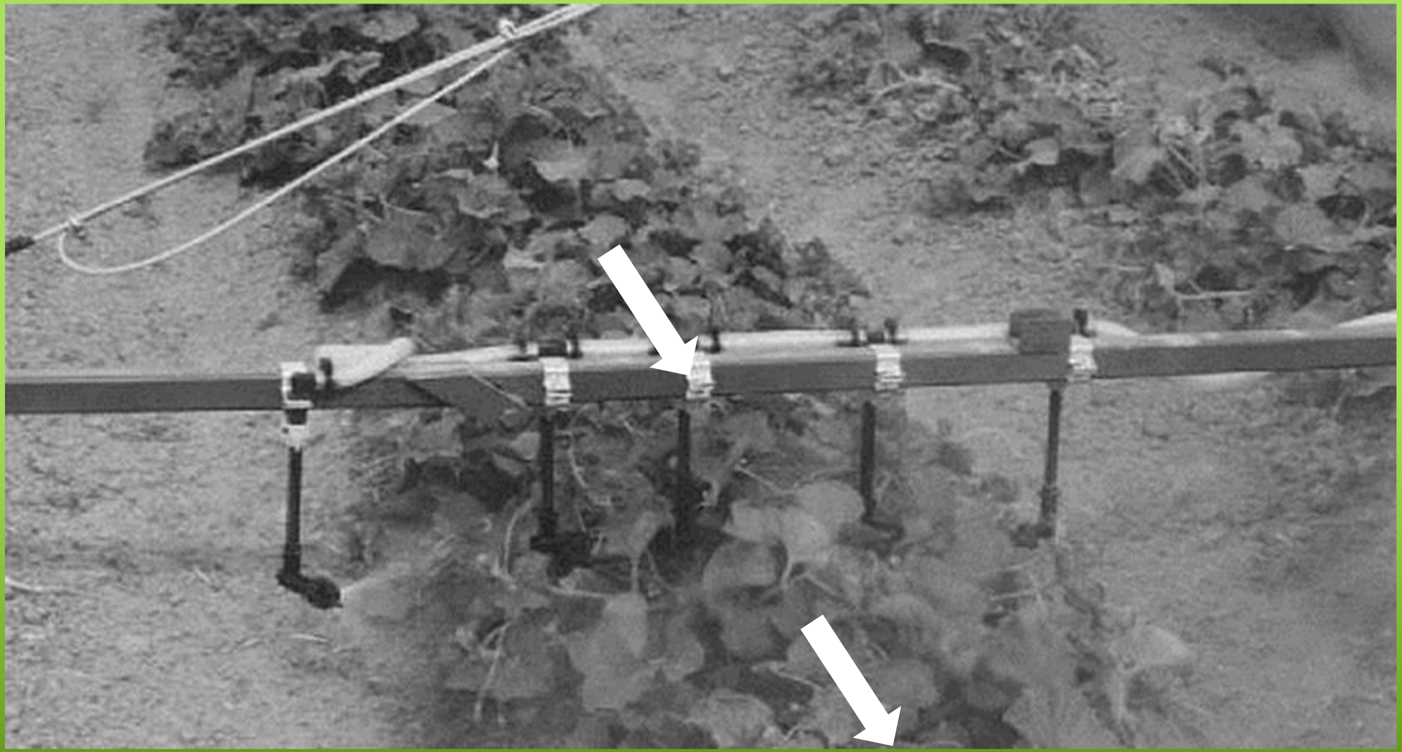
What I learned from one poinsettia grower, for whitefly:

- Arrange plants in elevated rows
- Spray from below, nozzles on both sides

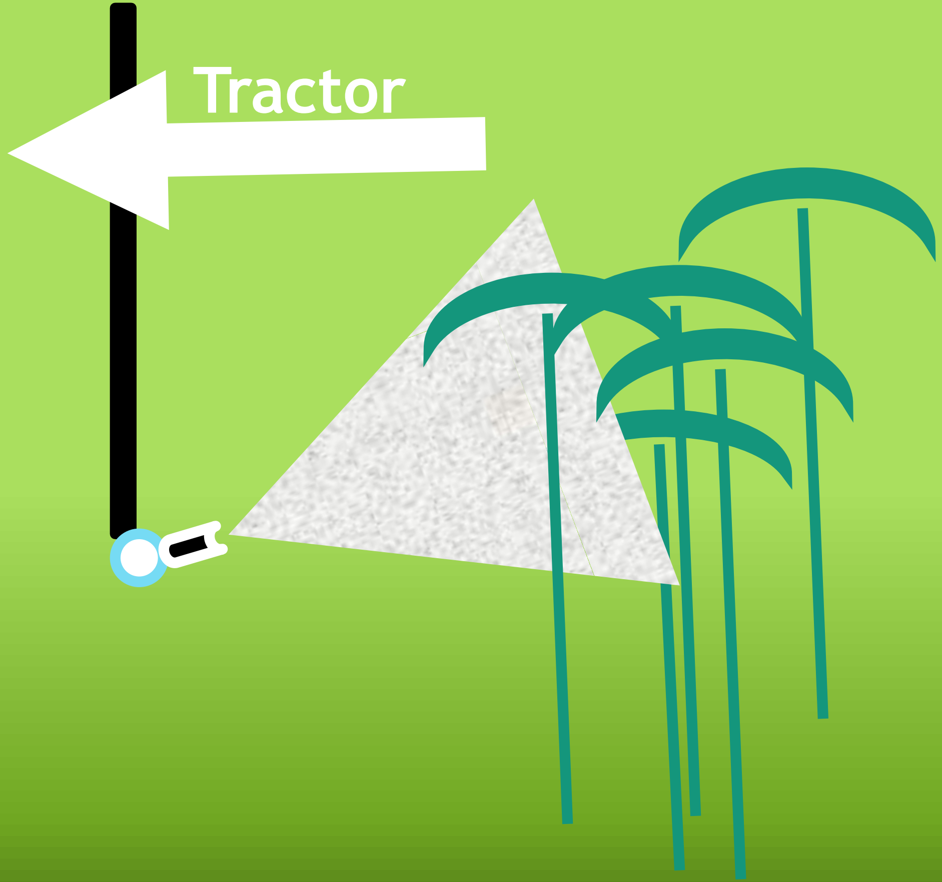
Coverage, coverage, coverage

Creative approaches in application to improve coverage sometimes needed

Spraying leaf undersides to control whitefly using drop nozzles at canopy level, directed <u>horizontally</u> and <u>backwards</u>

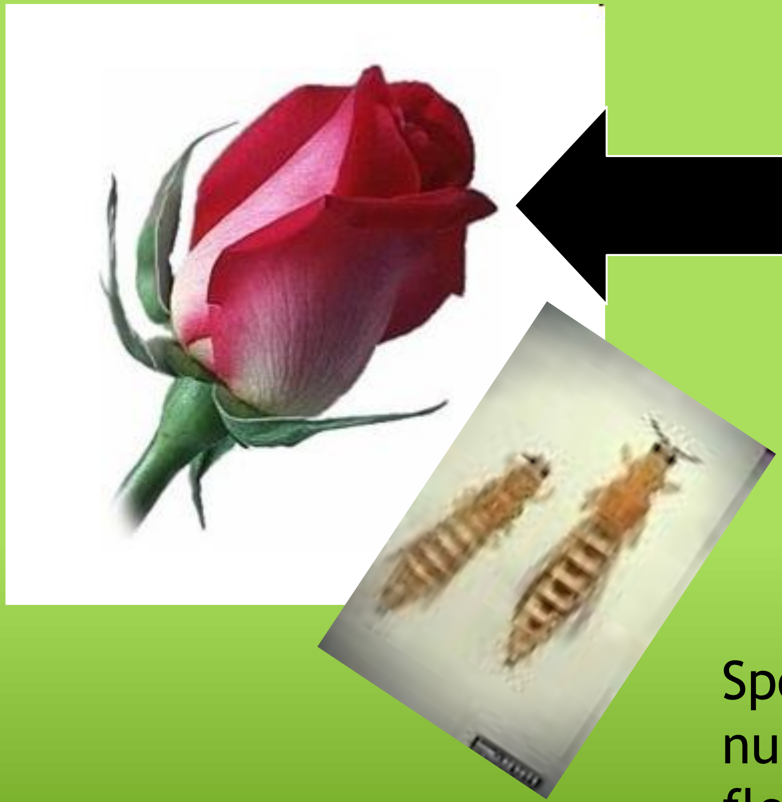


Jaronski 2010



Coverage, coverage, coverage

One can use additives, adjuvants, to microbe formulations to make them work better.



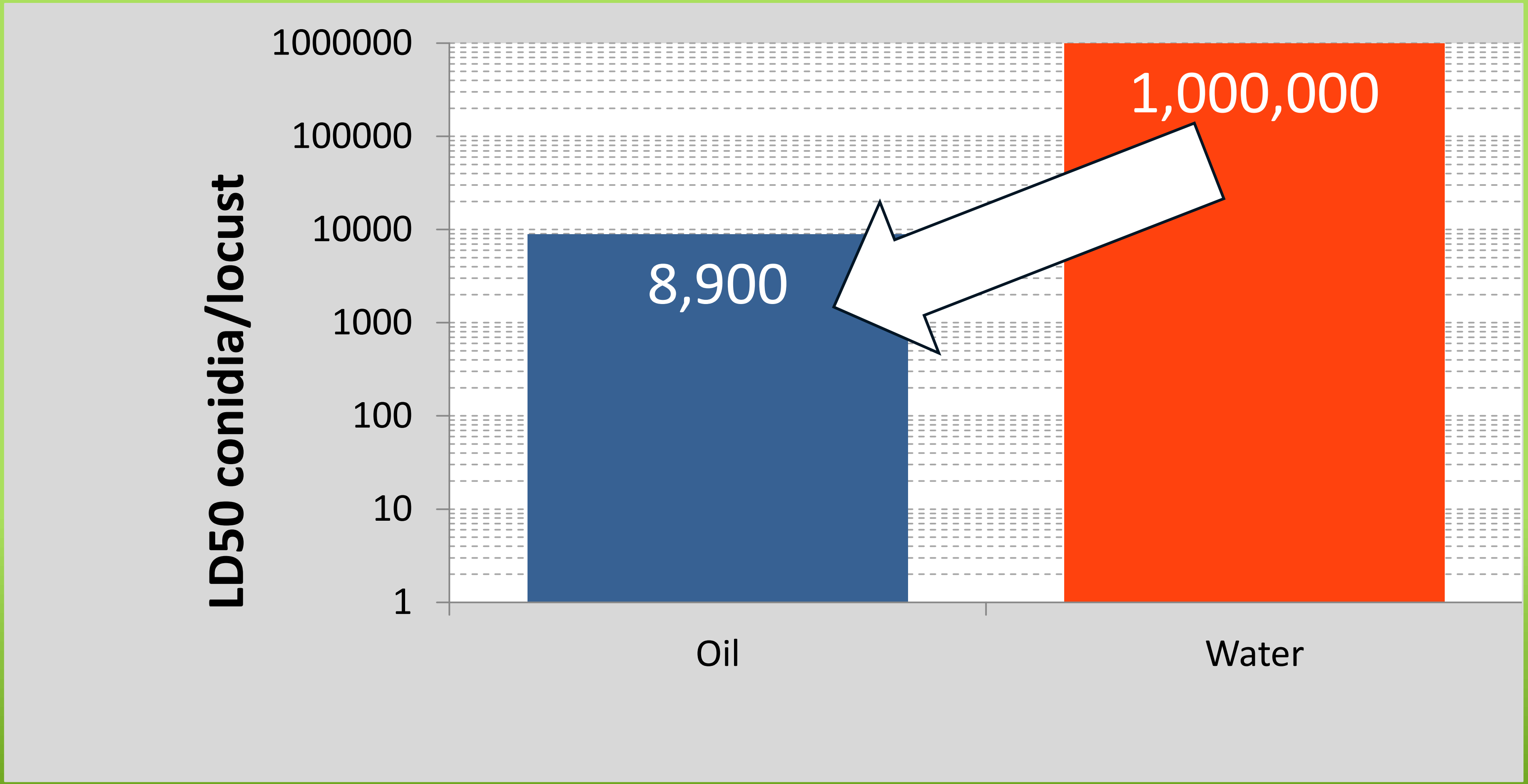
BotaniGard 22WP
+ 0.06% Silwet L77®

Spores penetrated in substantial numbers into 5-6th petals of unopened flower

Control much better than 22WP alone

The mycoinsecticide formulation can help — Neat oil, oil-in-water emulsions, can be better than wettable powders

Oil-based formulation increases efficacy



Bateman et al . 1993. Annals Appl Biol

Mycoinsecticide "ES" ("EC", "OD") versus WP…
It depends. On the insect, and the stage of insect

Effect of Bb GHA Formulation on Efficacy
(and effect of insect stage on susceptibility)

	Efficacy			
	Western Flower Thrips		Cotton Aphid	
Formulation	Adult	Larva	Adult	Nymph
ES	58 ±11%	**82 ±2%**	**98 ±3%**	38 ±9%
22WP	**88 ±6%**	**90 ±0%**	62 ±2%	38 ±10%
(Untreated)	0%	0%	0%	0%

ES: 0.5 qt/100 gal; 22WP: 1 lb/100 gal = 1E13 conidia

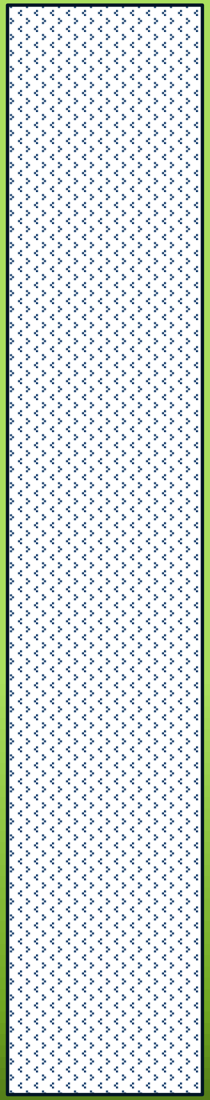
B. Murphy, unpublished data

Take advantage of target insect's behavior to increase transfer efficiency:

- Put the spores where larvae (e.g. cherry fruit fly) fall to pupate in soil beneath trees create a 'bio minefield'

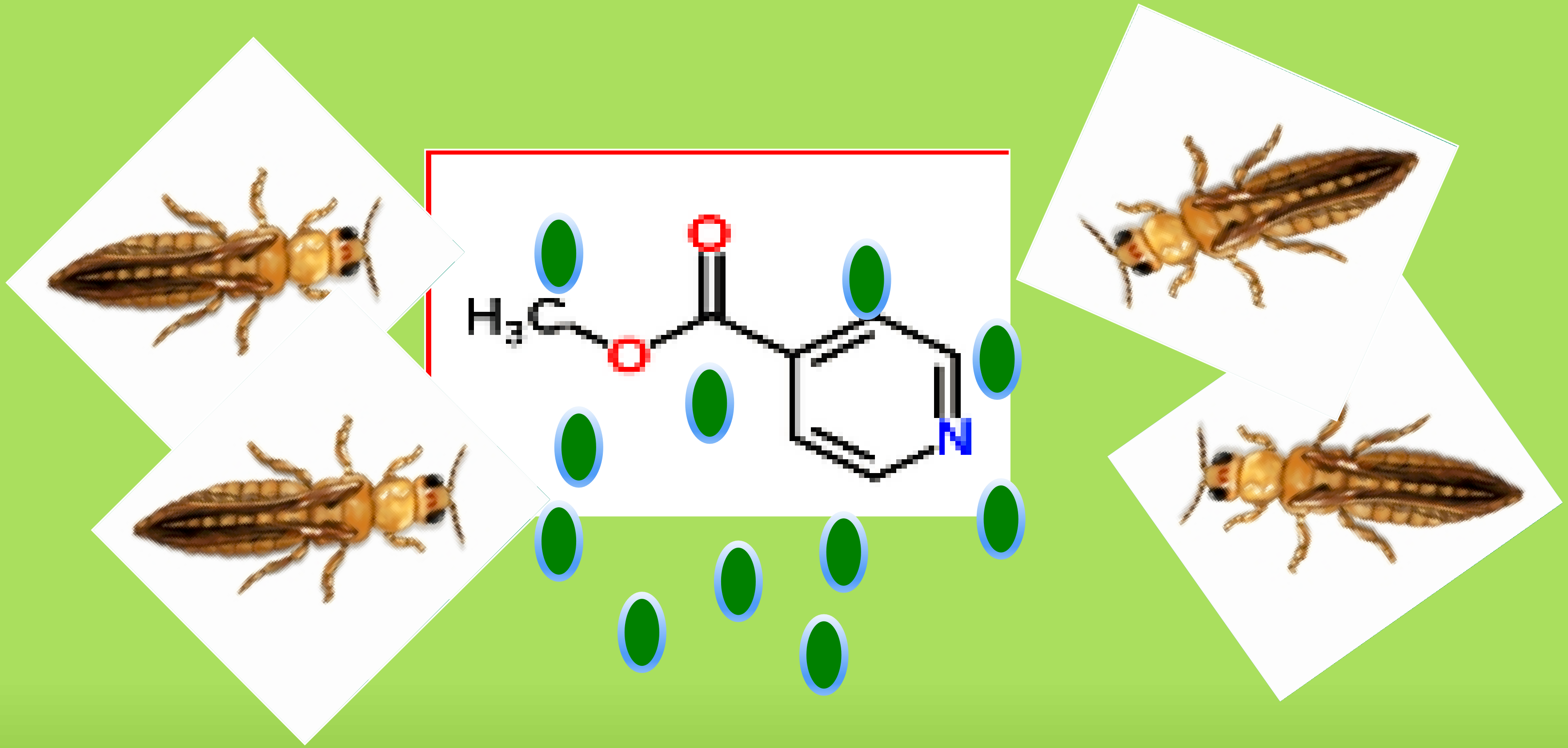
Cossentine and Jaronski

Take advantage of target insect's behavior:
Beauveria for bedbugs - 2-3 inch barrier of spores (in an oil) between bedbug and its food (people)



Courtesy ConidioTec

Or, Bring the insect to the microbe

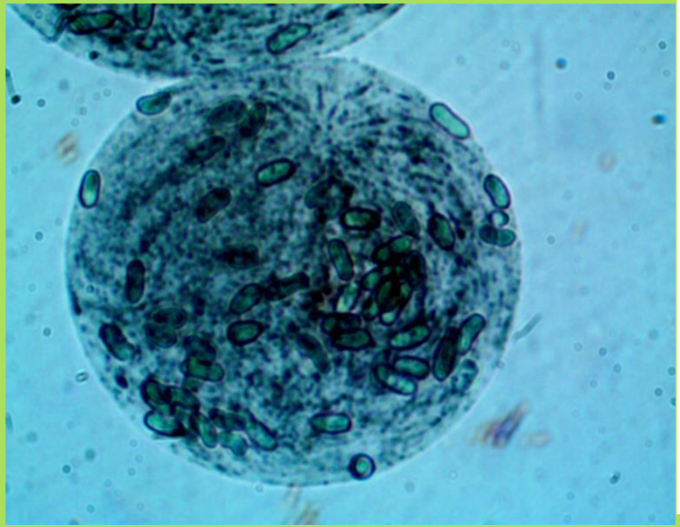


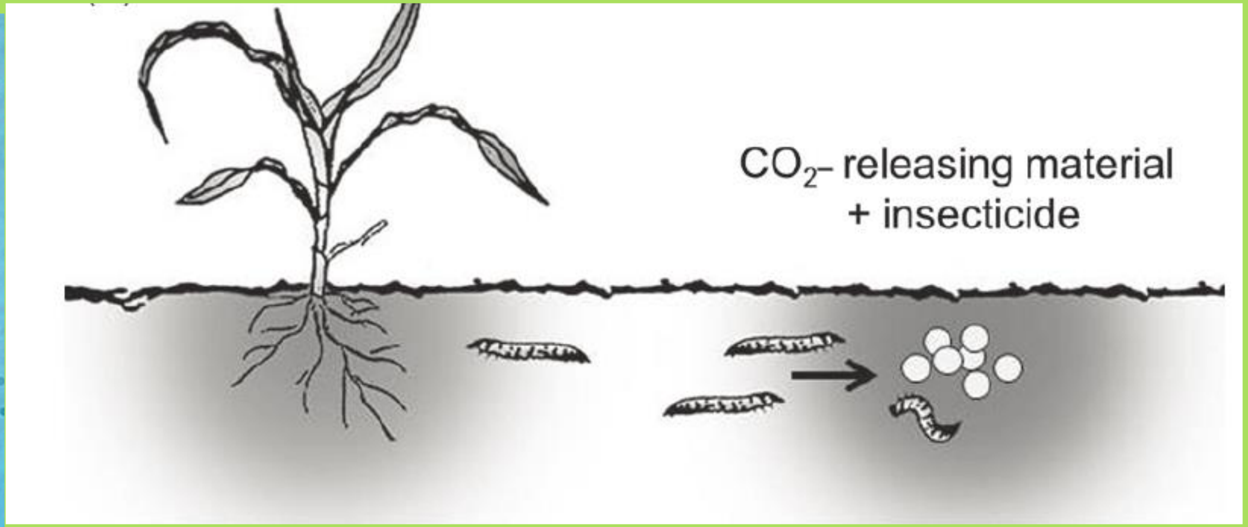
Metarhizium applied with methyl-isonicotinate, Lurem-TR, to attract thrips to conidia.

Note: approach not on any mycoinsecticide label US or Canada

Niassy et al 2011. Entomologia Experimentalis et Applicata

Bring the insect to the microbe



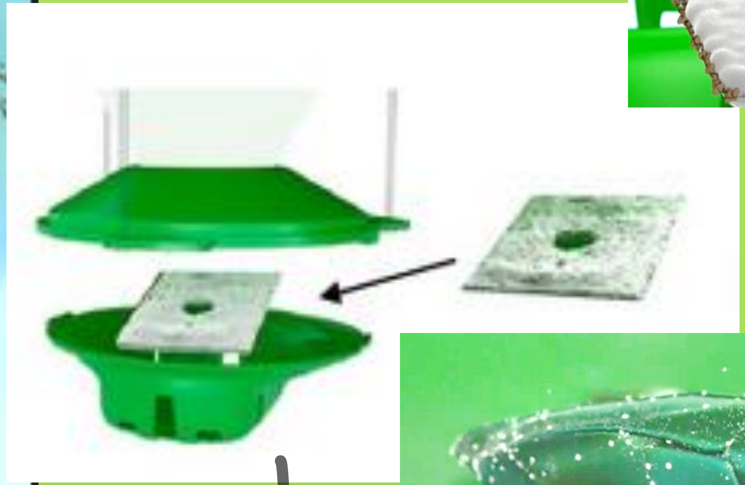


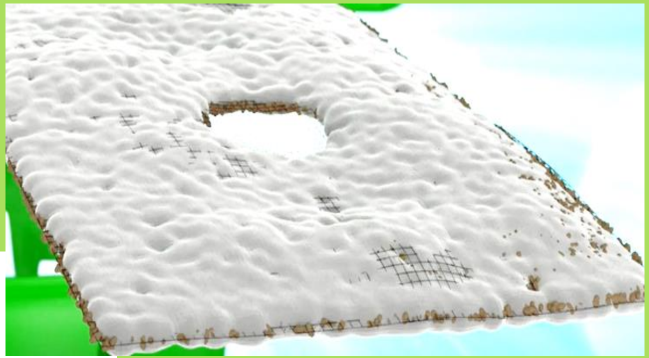
- Alginate beads with fungus spores <u>and CO_2-releasing yeast</u> to attract wireworms to a "Fatal Candy"

… commercial in Germany

Vemmer et al 2016

Bring the insect to the microbe





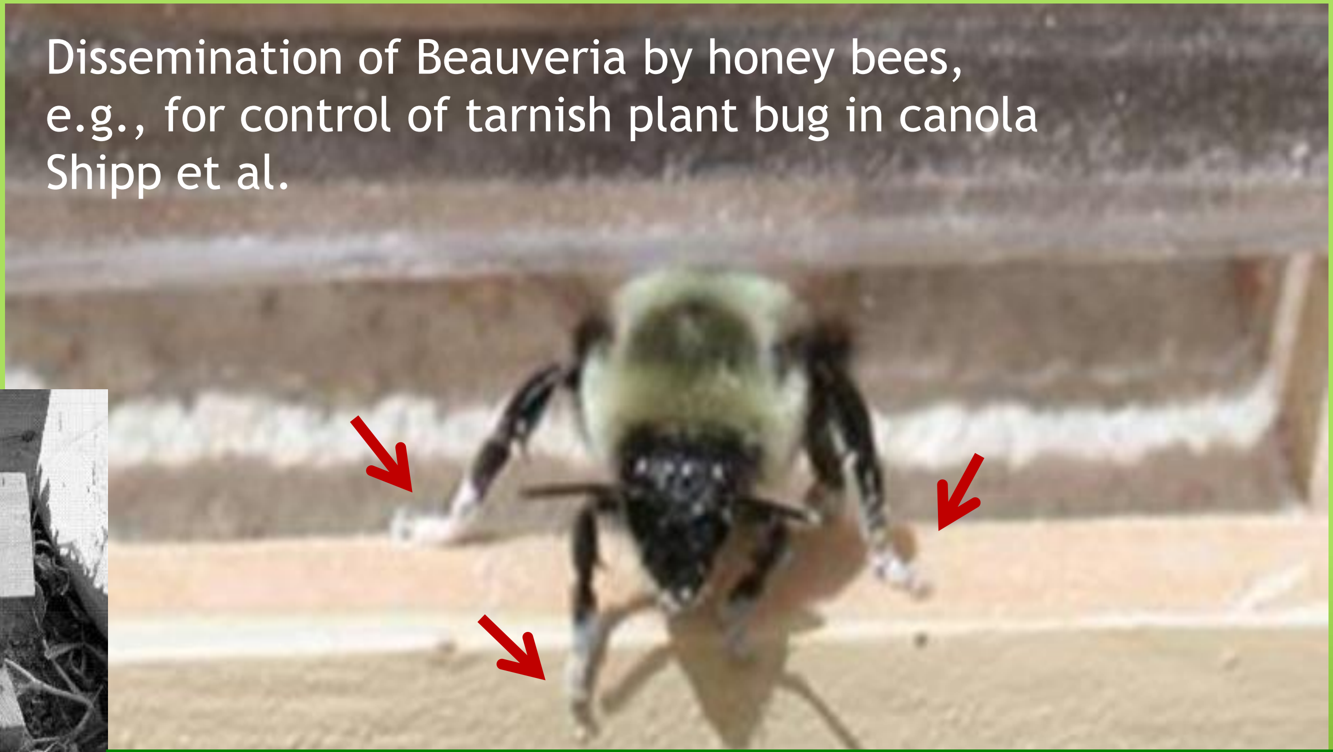
Pad of sporulated Beauveria

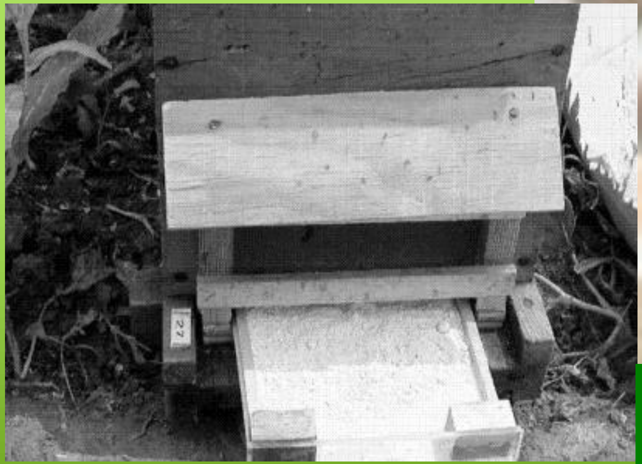
Fraxiprotec® for Emerald Ash Borer

(Canada, soon US)

Use another insect to transfer the microbe to where it's needed

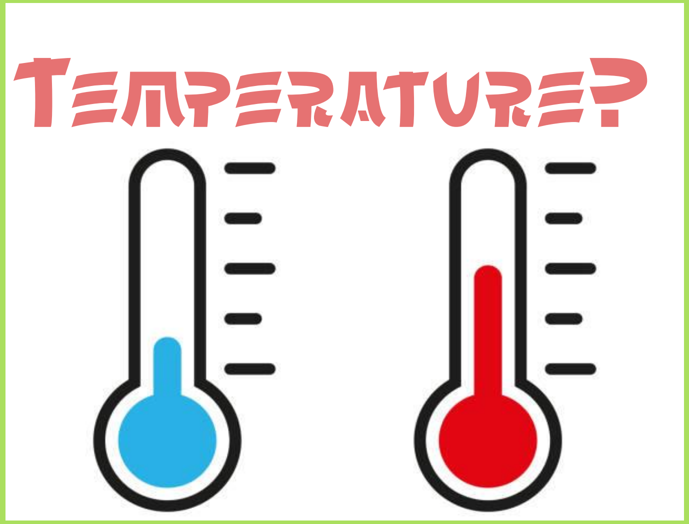
- Honeybees vectoring *Beauveria fungus* to flowers



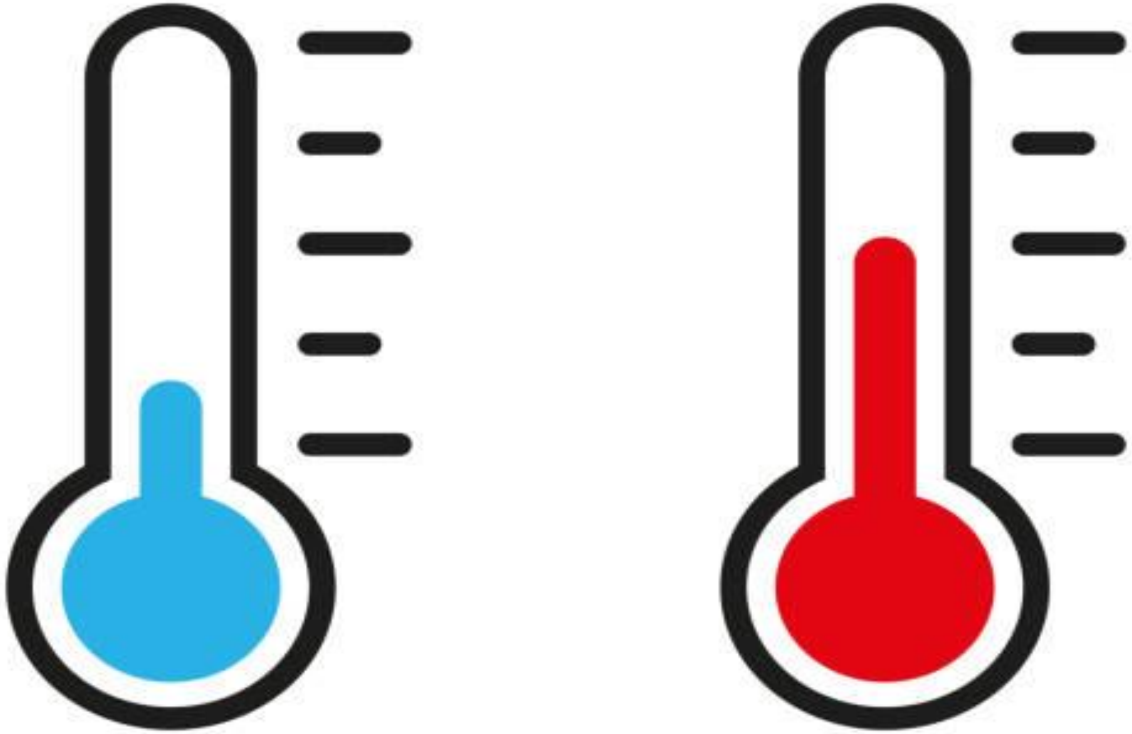


➔ **BotaniGard 22WP for bee vectoring in Canada, not US**
➔ Biobest "Flying doctors" program for other fungi
➔ Bee Vector Technologies system for other fungi

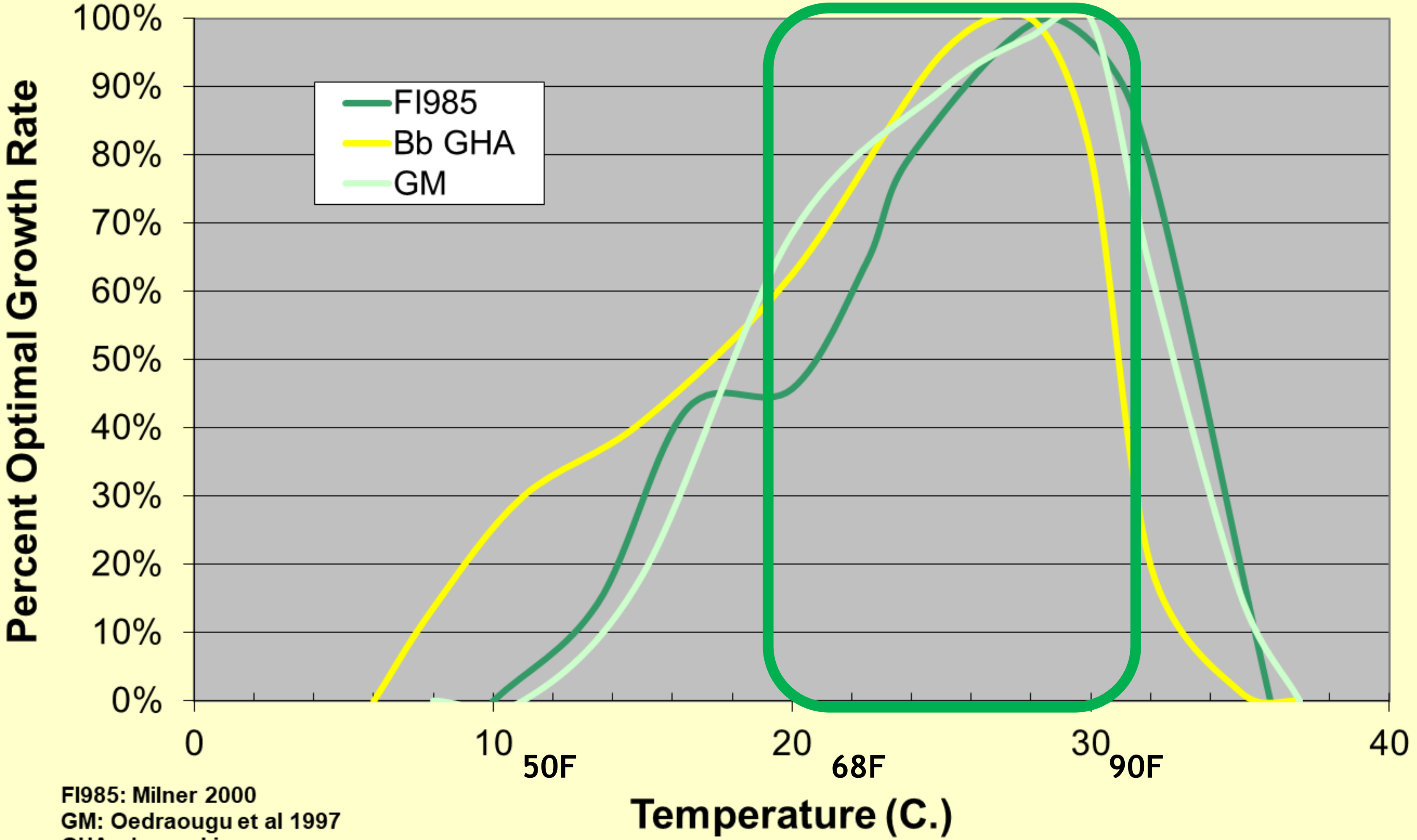
But what about effect of



Temperature?

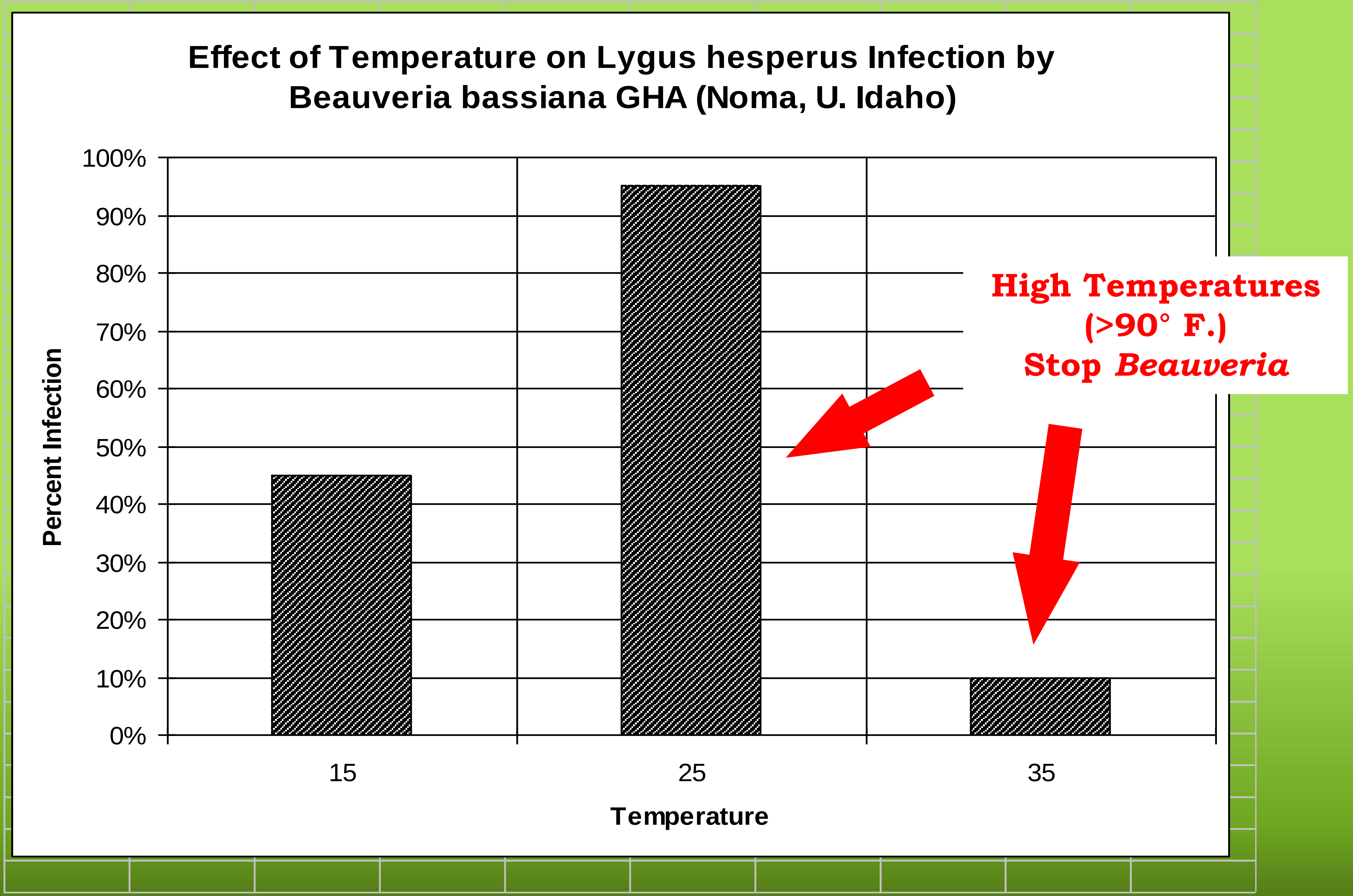


Thermal Tolerances of Bb GHA, Meta FI-985, Meta Green Muscle (GM)



FI985: Milner 2000
GM: Oedraougu et al 1997
GHA: Jaronski

EFFECT OF TEMPERATURE …



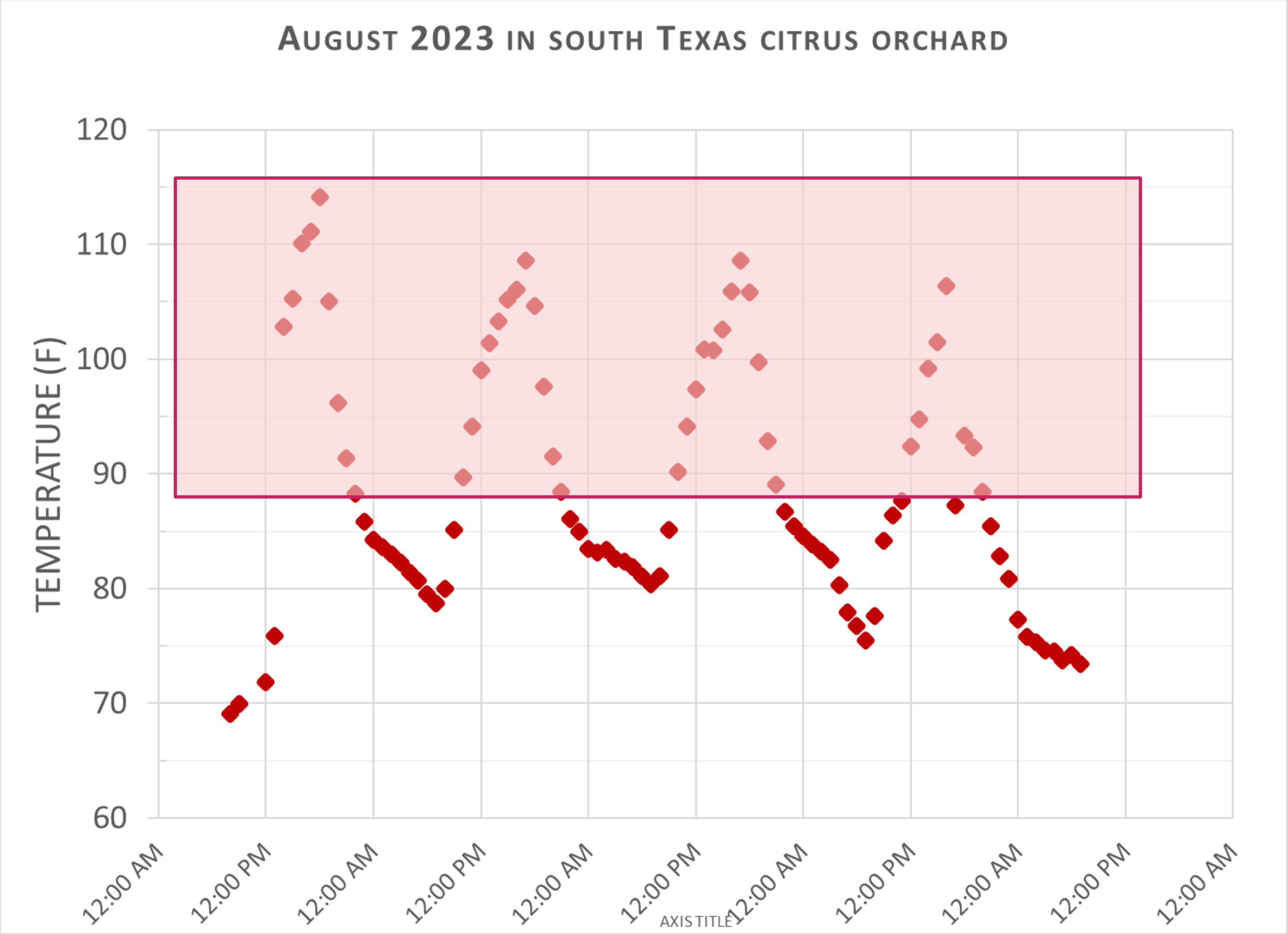
Another example: *Beauveria* for Asian Citrus Psyllid

% Mycosis
61.2%
52.1%
27.3%
30.3%
9.2%
9.1%

Another example: *Beauveria* for Asian Citrus Psyllid

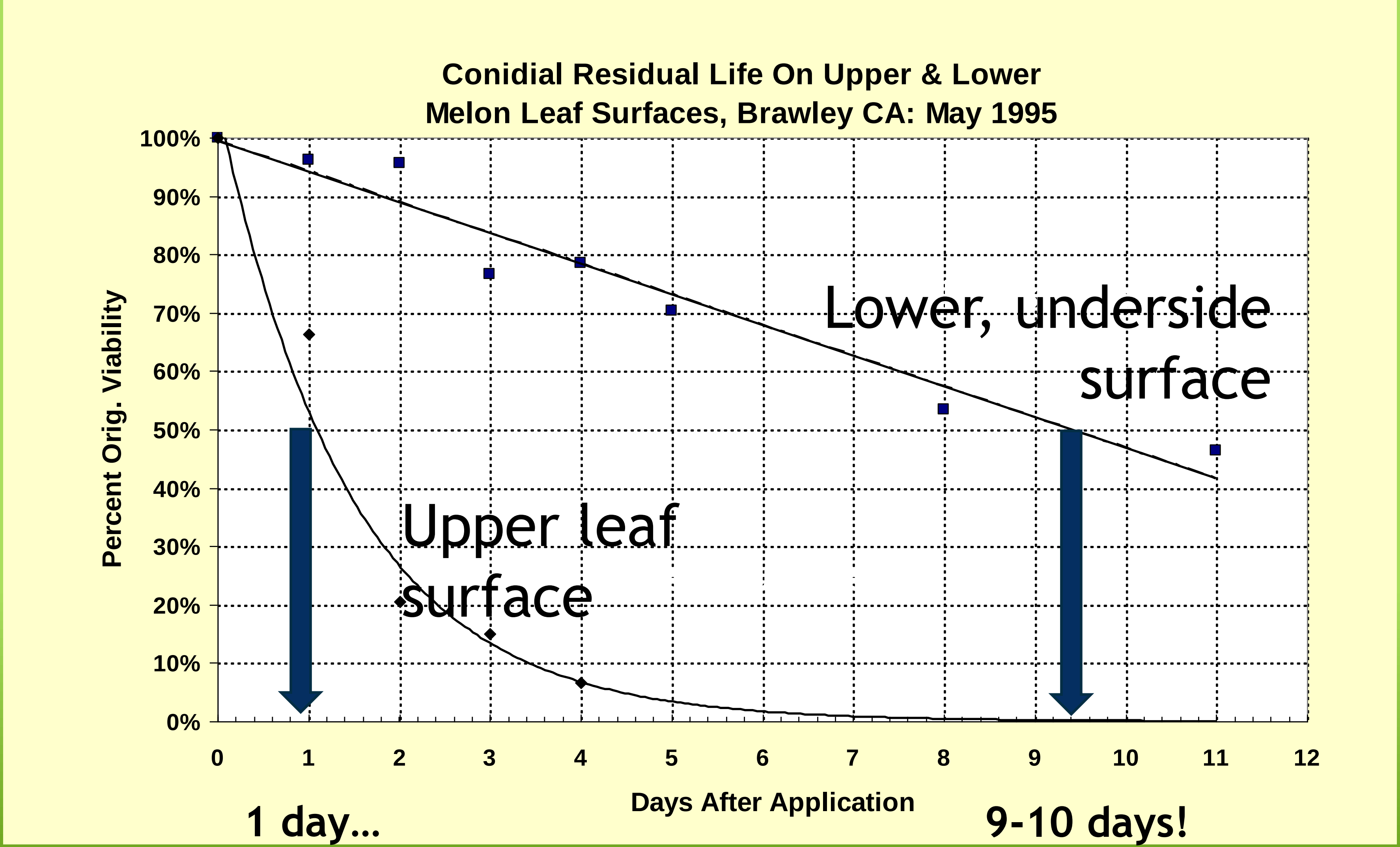
% Mycosis	Temp Hours <20C	**Hours >32C**	Total Hours
61.2%	43	0	96
52.1%	40	0	97
27.3%	44	**4**	95
30.3%	44	**4**	95
9.2%	0	**11**	95
9.1%	0	**18**	95

Moral of the lesson: Do not use Beauveria in the hot summer (e.g. south Texas) …



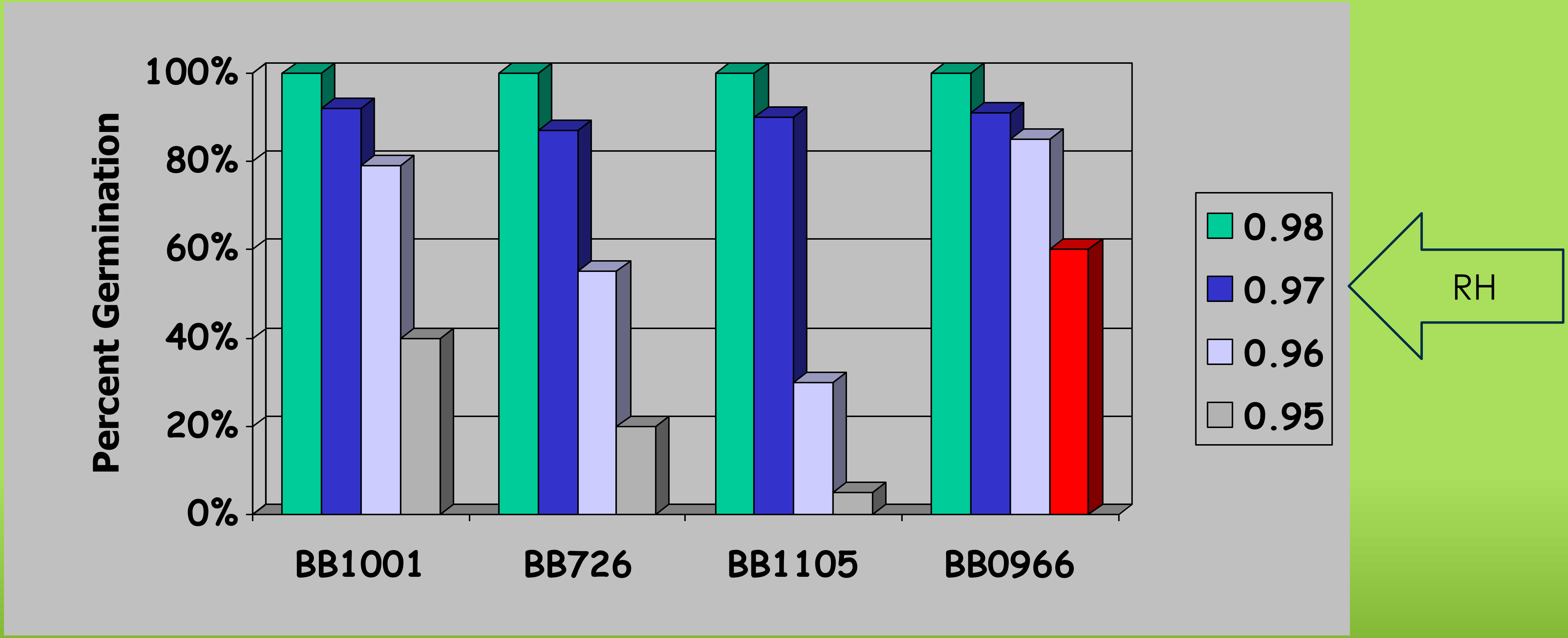

Sunlight (UV)?

Sunlight (UV-A/UV-B) kills spores …




Humidity?

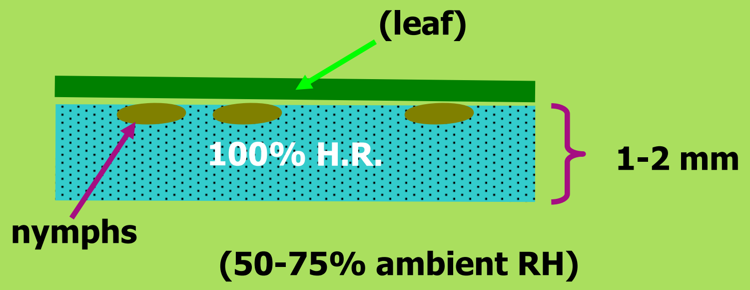
Spore germination does require high humidity



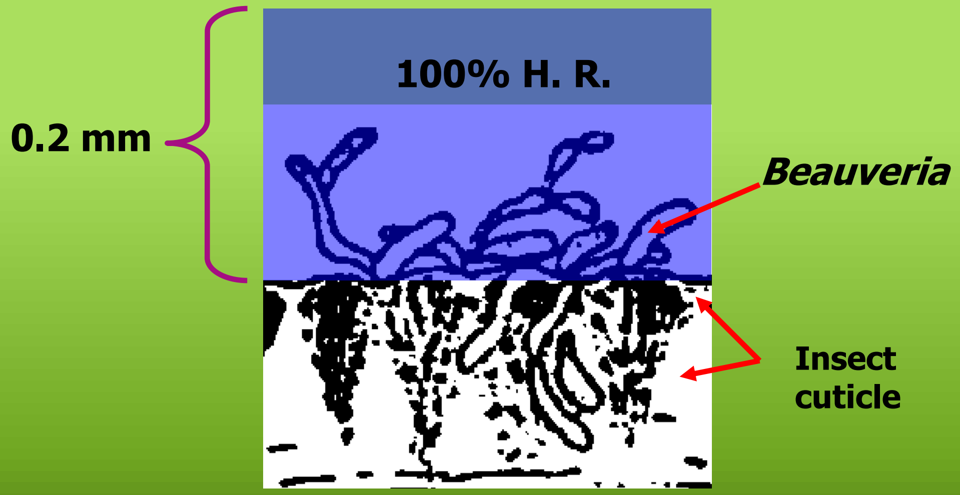
But,
Ambient Humidity can have little relevance

Why?

For small insects (whitefly nymphs) the humidity of the leaf surface is high enough



Cuticle of many larger insects has layer of high humidity



Humidity had little effect (whitefly nymphs)

Mortality of *Bemisia argentifo* *Beauveria bassiana* and *Paecilomyces fumosoroseus*
and Incu oisture Conditions at 23 ± 2°C

Fungus/strain	(via	Mean % mortality ± SE[a]	
		Incubated 24 h at 100% RH then 8 days at 49–54% RH	Incubated 9 days at 49–54% RH
Test 1			
P. fumosoroseus ARSEF 3594[b]		67.7 ± 2.80	55.8 ± 8.95
B. bassiana ARSEF 252[b]		75.1 ± 4.07	73.6 ± 9.72
Spray control (0.01% Tween 80)		8.1 ± 2.74	2.7 ± 0.30

In summary,

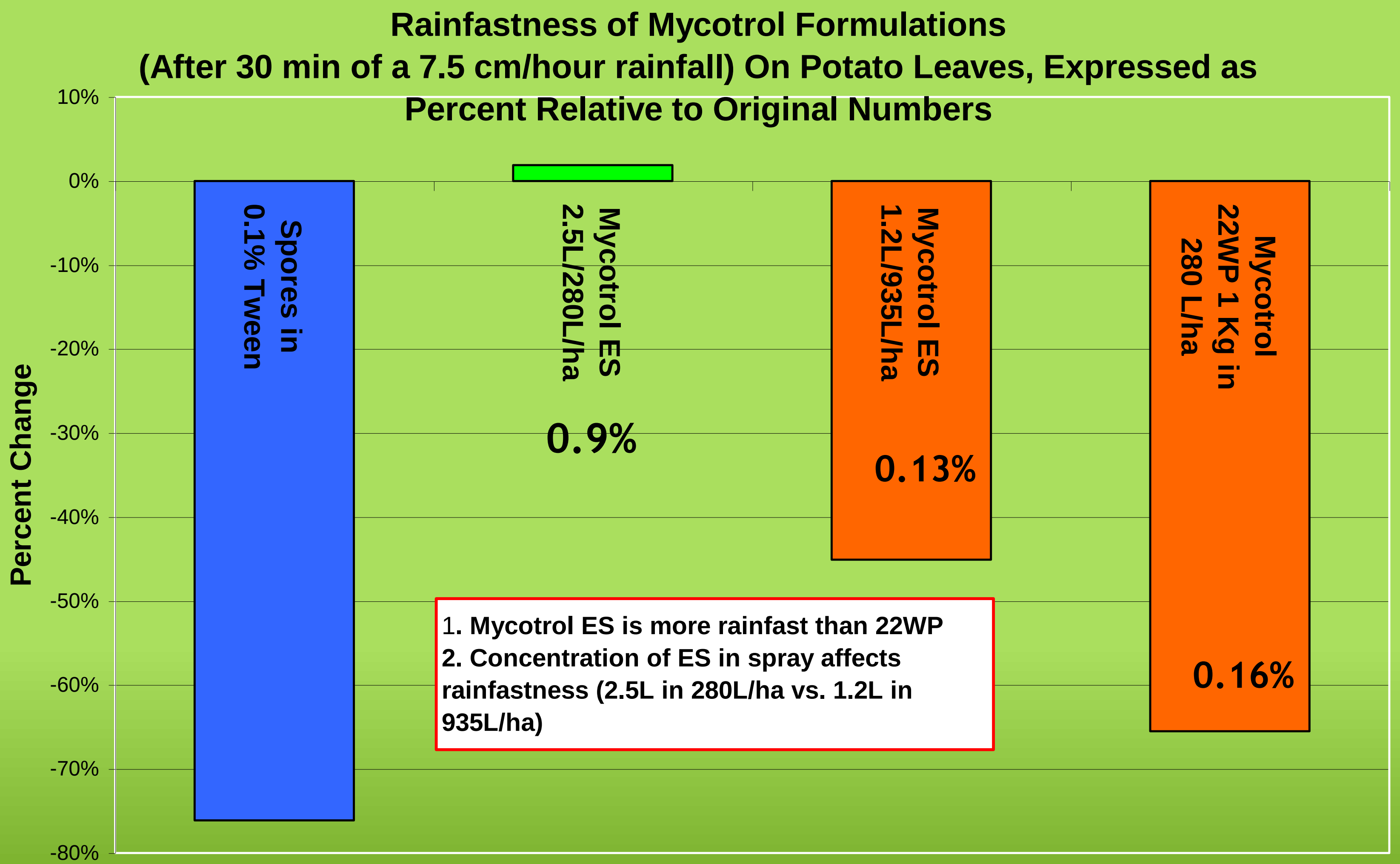
High <u>ambient</u> relative humidity,

- is **NOT required for infection**
  except for large insects in exposed places, or when wind is present
  (humidity on leaf surface or insect surface is usually high enough)
- Oil-in-water (EC, ES) spore sprays avoid low RH effects

- **IS required for sporulation** and recycling
  IF one wants that…


Rain?

Rainfastness is Affected by Formulation and Rate



Inglis et al. 2000

ES, EC formulations can have greater rainfastness than WPs, esp. when applied in greater concentrations e.g. 0.9% vs 0.14%

FUNGICIDES . . . ?

What about compatibility with fungicides?

TANK MIX	Brand Tested	BotaniGard ES Compatibility
Ampelomyces quisqualis	AQ10®	Tank-mix OK
Copper Lineolate	Tenn-Cop 5E®	Tank-mix OK
Copper Hydroxide	Kocide DF®	Tank-mix OK
Copper Sulfate	Phyton 27®	Tank-mix OK
Thiophanate-methyl	Cleary's 3336 WP®	Tank-mix OK
Fosetyl-Al	Aliette WDG®	Tank-mix OK
SAME DAY		
Sulfur - volatilized	various	Same Day

What about compatibility with fungicides?

Many are compatible, when spaced apart from the fungus.

Check with the specific manufacturer of the mycoinsecticide

Fungicides

Brand Name	Active Ingredient	Maximum Tested Rate	Compatible
Actinovate SP®	*Streptomyces lydicus* WYEC 108	24 oz/100 gal	Yes
Acrobat®	Dimethomorph	20 oz/100 gal	Yes
Aliette WDG®	Fosetyl-Al	5 lb/100 gal	Yes
Alude®	Mono- and di-potassium salts of phosphorous acid	12.75 oz/100 gal	4 Days B/A[2]
AQ10®	*Ampelomyces quisqualis*	3.3 oz/100 gal	Yes
Banner Maxx®	Propiconazole	16 oz/100 gal	2 Days B/A[2]
Banol®	Propamocarb	4 lb/100 gal	4 Days B/A[2]
Bayleton®	Triadimefon	20 oz/100 gal	2 Days B/A[2]
Bravo 720®	Chlorothalonil	10 pt/100 gal	4 Days B/A[2]
Captan® 4000	Captan	20 oz/100 gal	4 Days B/A[2]
CEASE®	*Bacillus subtilis* strain QST 713	8 qt/100 gal	1 Day B/A[2]
CEASE®	*Bacillus subtilis* strain QST 713	4 qt/100 gal	Yes
CEASE® + MilStop® + BotaniGard 22WP	*Bacillus subtilis* strain QST 713 + Potassium bicarbonate + *Beauveria bassiana* strain GHA	4 qt + 2.5 lb + 2 lb/100 gal	Yes
Chipco 26019®	Iprodione	2 lb/100 gal	2 Days B/A[2]
Cleary's 3336 WP®	Thiophanate-methyl	6 lb/100 gal	Yes
Combine®	Bromoxynil	3 gal/100 gal	Yes
Copper Count-N®	Copper ammonium carbonate	3 qt/100 gal	2 Days B/A[2]
Curalan®	Vinclozolin		2 Days B/A[2]
Daconil®	Chlorothalonil	2 qt/100 gal	4 Days B/A[2]
Decree®	Fenhexamid		Yes
Dithane F-45®	Mancozeb	8 qt/100 gal	4 Days B/A[2]
Eagle®	Myclobutanil	16 oz/100 gal	2 Days B/A[2]
Ecogard GN®	*Bacillus licheniformis* strain 3086, indole-3-butyric acid	64 fl oz/100 gal	4 Days B/A[2]
Elevate® 50 WDG	Fenhexamid		Yes
Endorse®	Polyoxin D zinc salt	2.2 lb/100 gal	4 Days B/A[2]
Fenstop®	Fenamidone	28 fl oz/100 gal	Yes
Flint®	Trifloxystrobin		2 Days B/A[2]
Folicur®	Tebuconazole	7.7 fl oz	4 Days B/A[2]
Funginex®	Triforine	50 oz/100 gal	2 Days B/A[2]
Heritage®	Azoxystrobin	40 oz/100 gal	4 Days B/A[2]
Hurricane®	Mefanoxam and fludioxonil	1.5 oz/100 gal	4 Days B/A[2]
Insignia®	Pyraclostrobin	16 oz/100 gal	4 Days B/A[2]
Kocide ® 3000	Copper Hydroxide	16 lb/100 gal	Yes
Luna Privilege®	Fluopyram		Yes

Can Resistance Develop???

Can Resistance Develop???

It is unlikely:

- Fungus is *active* agent, flexible, unlike molecule – no lock and key as with Bt or chemical
- Insect would have to evolve significant change in cuticle surface, or in humoral defenses (which, in general are not that strong)
- Some stink bugs, ticks, have evolved fungistats on cuticle – *long evolution*
- There is some data for *Drosophila* populations having different tolerances to a *Beauveria*, but at a very low dose (initial kill > 10 days, max kill at 25 days!)
- But no such differences among other insect populations e.g. Grasshopper populations to *Beauveria* (Jaronski unpublished).
- Several attempts to select for insect resistance to fungus unsuccessful.

The real problem: How too often have we been using these microbials?

As **CHEMICALS** …

Often <u>after</u> a full insect pest outbreak is present

And the microbes (fungi) do not work as well as chemicals

Microbes, fungi, ain't no magic bullets

How *can* these microbials be used best?

Not as a "fire extinguisher" against a full outbreak

But early, against the pioneer insects to PREVENT a pest outbreak

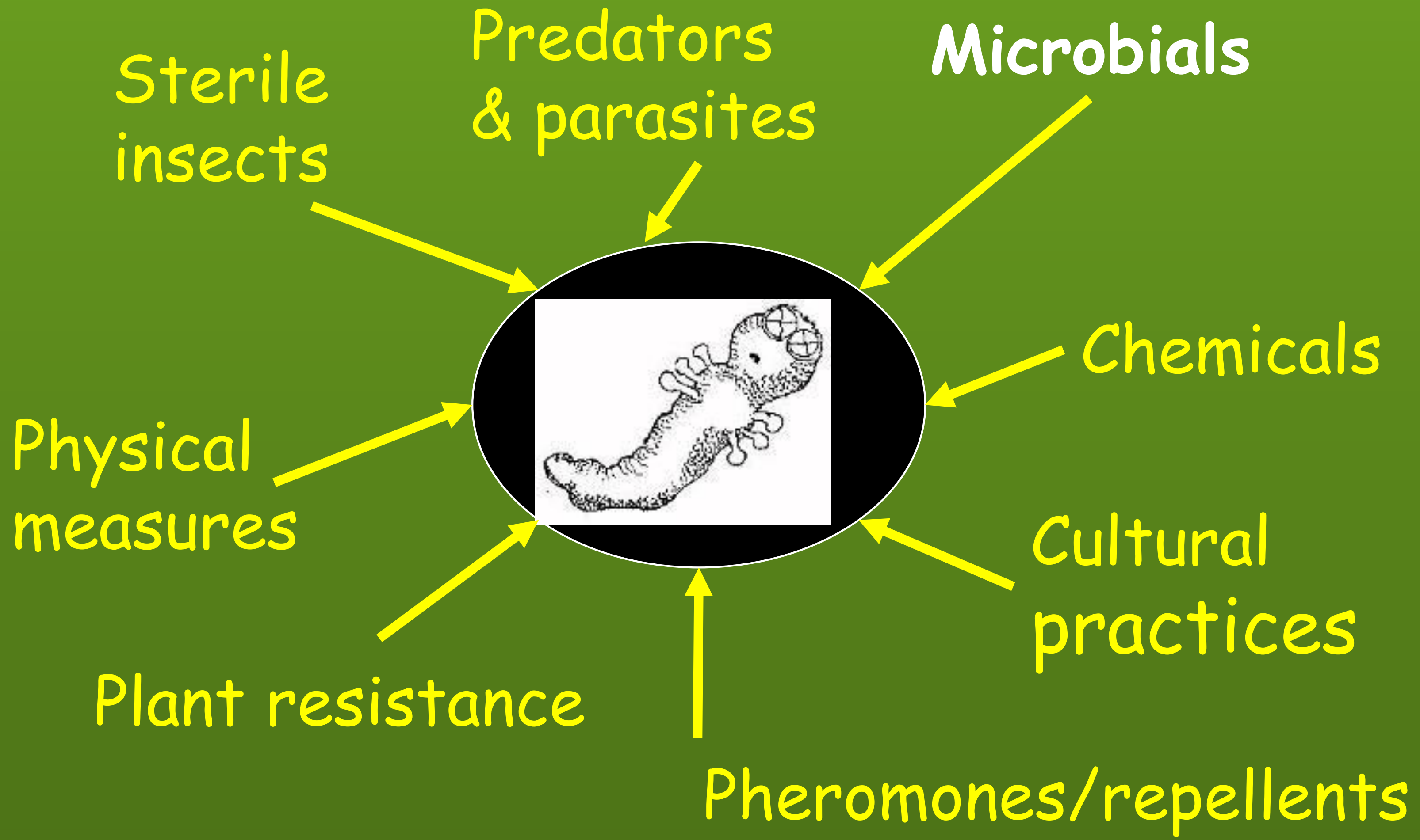
Total Control

versus

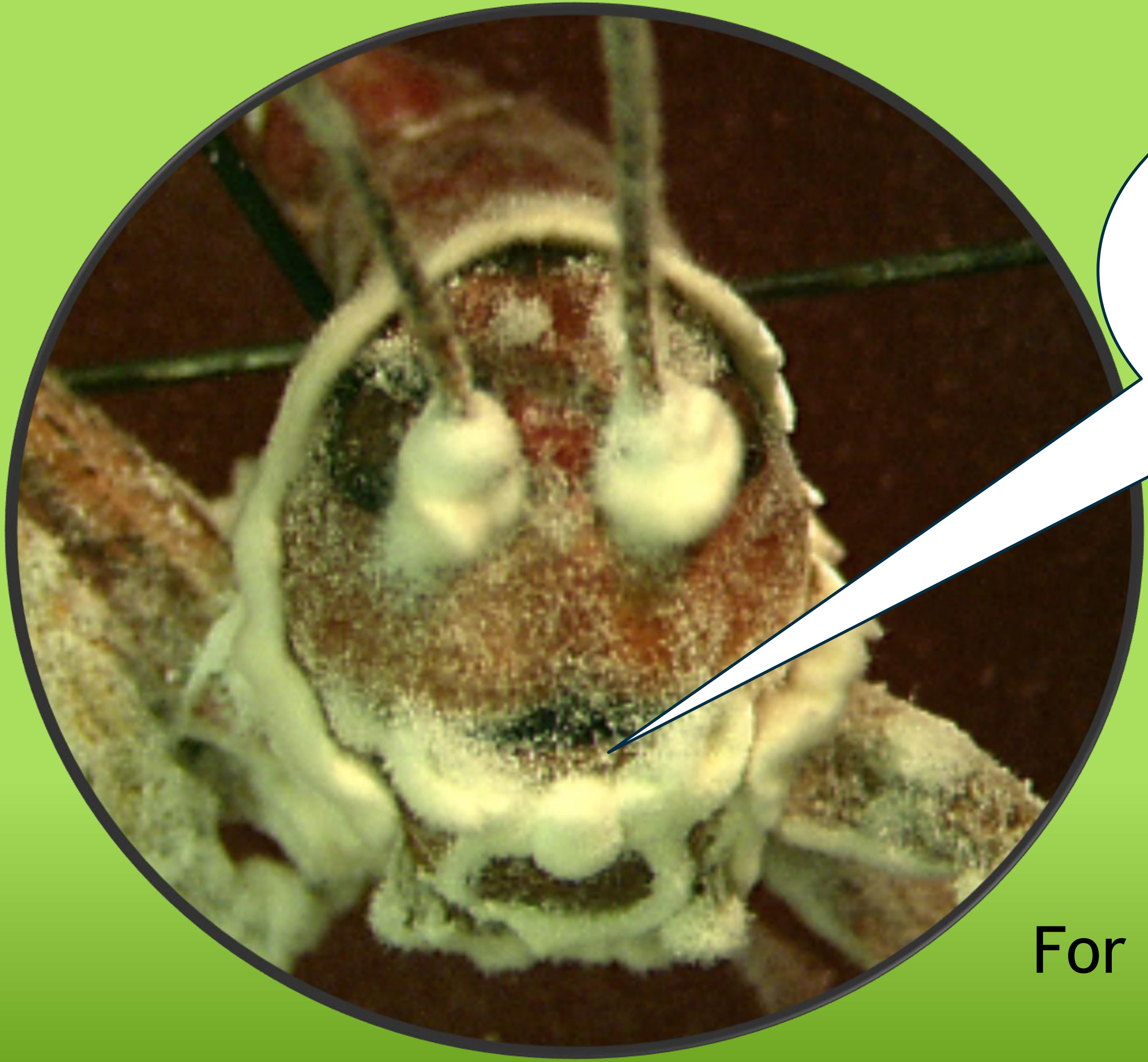
Management

to below economic threshold

Integrated Pest Management



Many tools used as a *system*



Thanks for your attention!

For more information, write to thebugdoc01@gmail.com