BEAUVERIA AND OTHER FUNGI: TOOLS *TO HELP MANAGE* COFFEE BERRY BORER, NOT MAGIC BULLETS

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What I hope to tell you today

1. Some basic information about these fungi
2. Issues facing successful use of fungi
3. *Thoughts about usefulness of Beauveria for CBB management (vs. control!)*

Entomopathogenic Ascomycetes / Hyphomycetes Our Cast of Characters

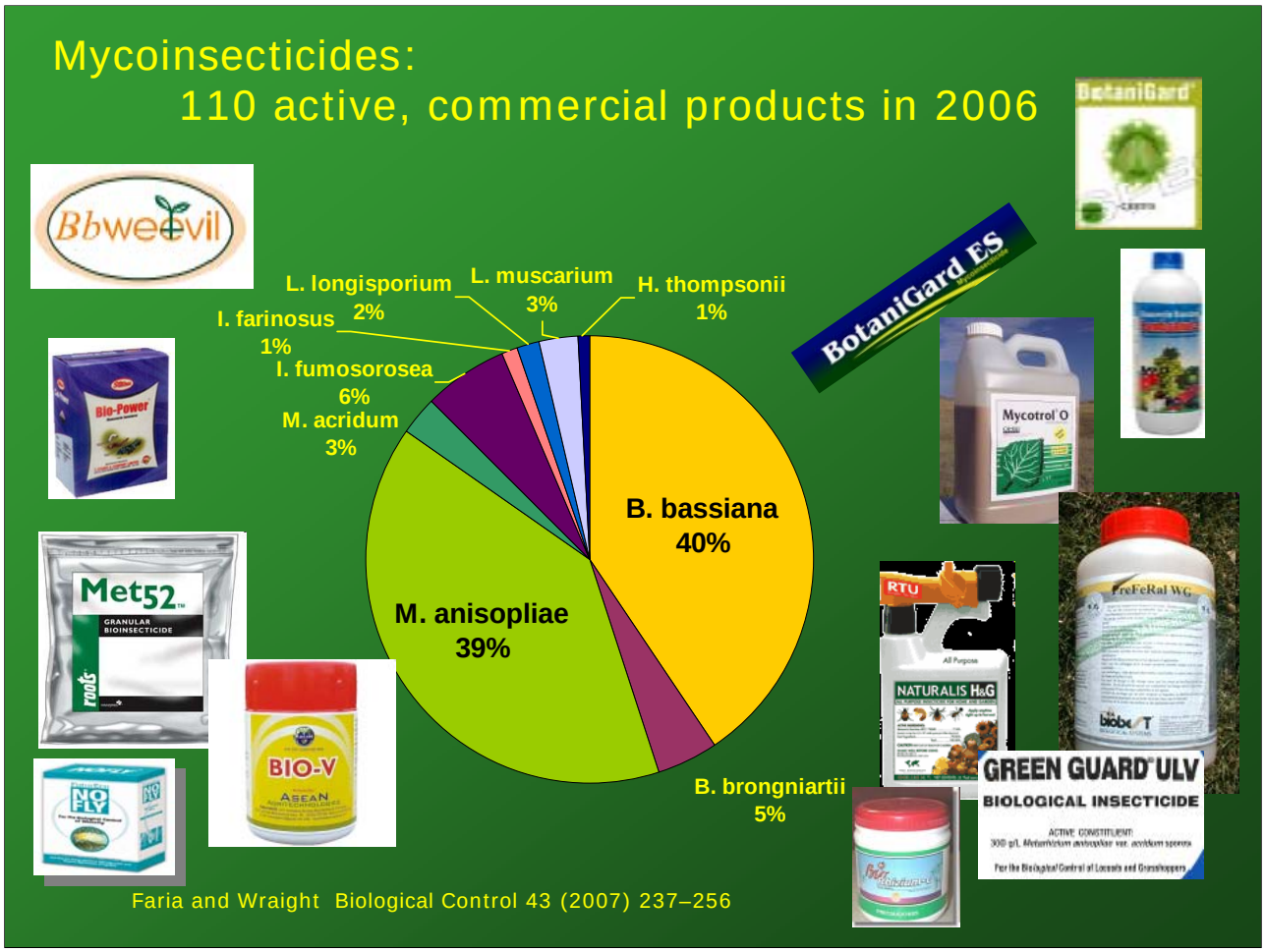
- *Beauveria bassiana & B. brongniartti*
- *Metarhizium anisopliae & M. acridum*
- *Lecanicillium longisporium, L muscarium, L sp. (Verticillium lecanii)*
- *Hirsutella thompsoni*
- *Isaria (Paecilomyces) fumosorosea & I. farinosus*
- *Nomuraea rileyi*
- *Aschersonia aleyrodis*

These fungi have been commercialized somewhere, at sometime.

This is the primary “cast of characters”

While historically all these fungi were classed in the Deuteromycetes, the Fungi Imperfecti, recent molecular tools have allowed scientist to associate these species with “perfect” stages all are the imperfect, assexual stages of Ascomycetes.

My comments today will be generally restricted to the fungus Beauveria bassiana (in white) because that is the one in which you are most interested.

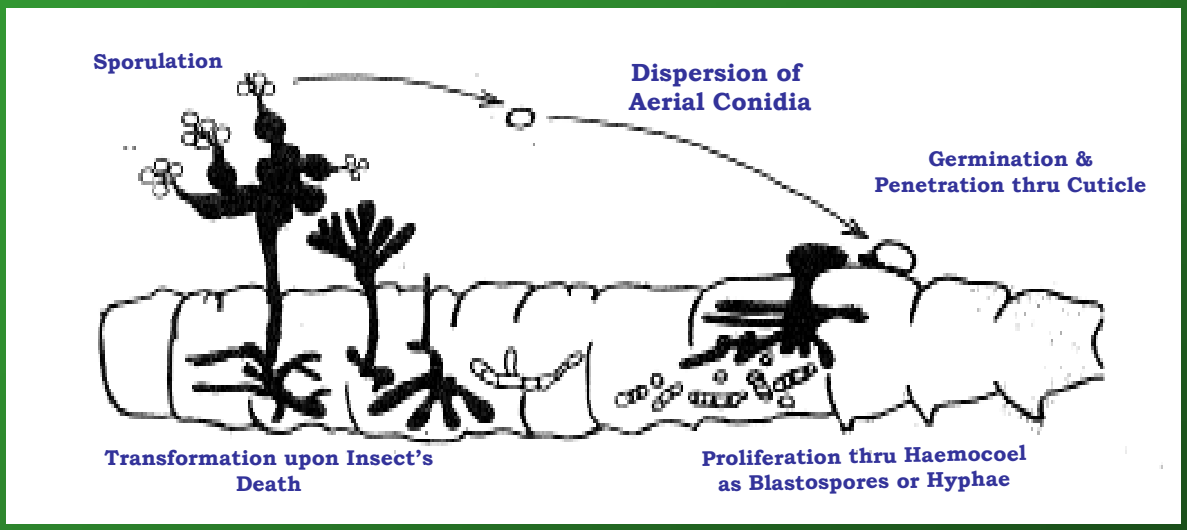


These fungi have been commercialized in a lot of countries and there are a lot of fungal products. In 2006 110 products were identified. Today there are probably closer to 150.

Beauveria and a related fungus Metarhizium represent the most common “mycoinsecticides.” Today.

In the U.S. there are two Beauveria strains and two Metarhizium strain registered by US EPA, but one Beauveria and one Metarhizium is really commercial.

Typical Life Cycle



The active ingredient of Beauveria and the others is the SPORE (aerial conidium). These fungi work like contact insecticides – spores have to contact the insect cuticle for the fungus to be effective.

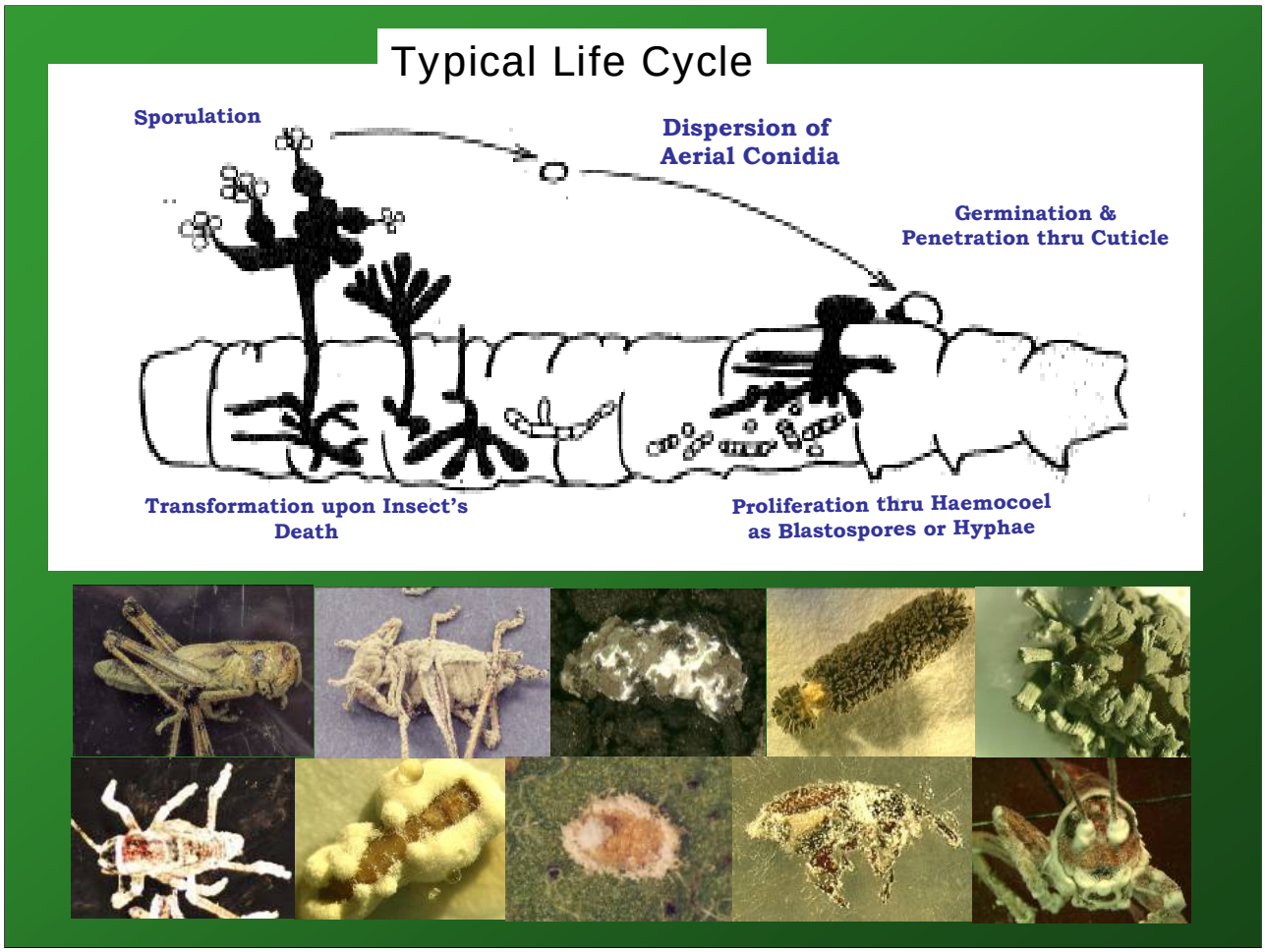
In a simple way, think "Fatal Athlete's Foot" of insects when you consider how these fungi work.

Key Events During Infection

1. **Attachment to Cuticle**
2. **Adhesion**
3. **Germination**
4. **Chemotaxis of Growing Hyphal Tip on Cuticle**
5. **Generation of Enzymes**
6. **Penetration**

When the spores come in contact with the insect, either from direct spray, or from the insect's habitat as it moves through it, they attach (via simple physical forces). The spores then "recognize" they are on insect cuticle, responding to chemical cues, and begin to germinate

The germinating spore produces an adhesive, binding it more strongly to the insect cuticle, swells and produces a growing tip (hyphal tip). This hypha then grows a wee bit on the cuticle then turns and penetrates into and thru the cuticle using mechanicla pressure, and a cocktail of enzymes. The spore germinates within 6-9 hours and the fungus penetrates into the interior of the insect within 24 hours. That's an important number to remember: 24 hours from the time the spore contacts the cuticle to the time it is inside the insect.



Once the fungus is inside the insect it grows as a yeast like phase throughout the insect's body, killing the insect within 3-10 days (depending on the dose of spores and the size of the insect).

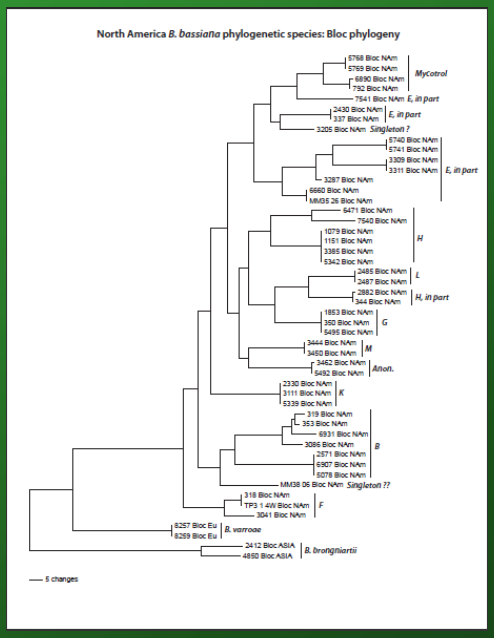
As the insect approaches death it is often mummified by the fungus. This is esp. true with caterpillars and other soft insects.

If condition are right (constant 96-100% relative humidity for a good 2-3 days), the fungus will emerge from the insect, cover it and produce millions of new spores. The tope row of photos are insects with the green Metarhizium fungus, while the bottom row shows Beauveria sporulating on various insects. This is easy to achieve in the lab, but rare in nature. In 30 years of working with this fungus coffee is one of the very few times I have seen it sporulate because there can be sufficient moisture.

In nature, the spores then disperse by wind and rain, and hopefully contact new hosts to infect.

Beauveria bassiana is genetically diverse

Beauveria (and *Metarhizium*, and the others) has tremendous genetic diversity

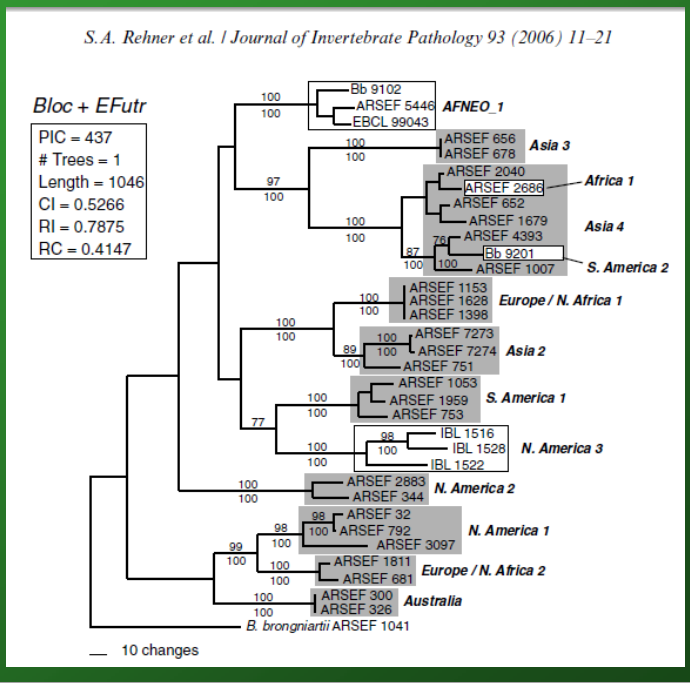


S. Rehner USDA ARS

Now, while you may think the Beauveria you see in dead borers is all the same, it is not. Beauveria (and the other fungi) is genetically very diverse. Here are th4e results of a study by an ARS molecular biologist about the diversity of Beauveria from North America. The longer the horizontal bar in this "tree" the more changes in the DNA sequence of just one gene (note bar in lower left indicating 5 changes in sequence).

"Sex" (genetic recombination) in Beauveria is rare. Thus mutations can accumulate in each line of fungus making it very different from others.

African and Neotropical Beauveria from CBB

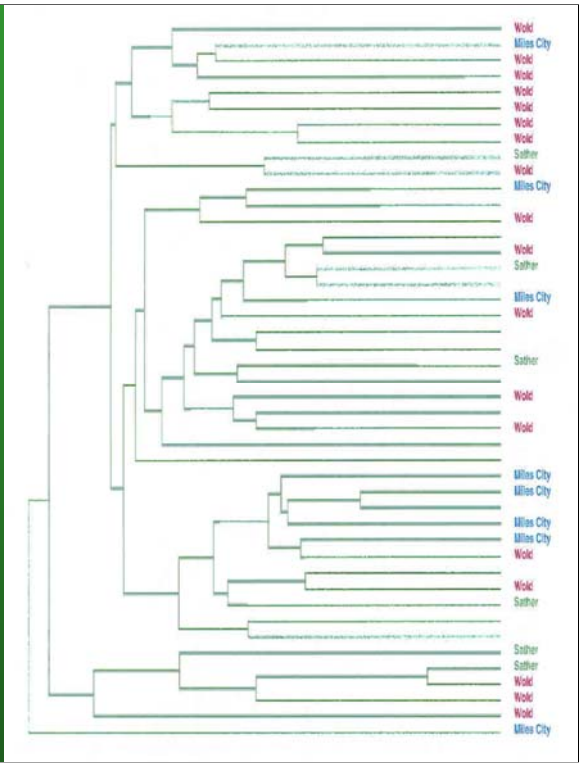


Here's an example of genetic diversity of Beauveria from just Coffee Berry Borer comparing strains from Africa and Latin America. Again the length of the horizontal bar in the tree indicates the number of changes in DNA sequence.

Note that some "haplotypes" from Africa, Asia and South America can be very similar despite their geographic origins. Beauveria bassiana is really a mixture of many strains, from every continent, with mixing over the world common.

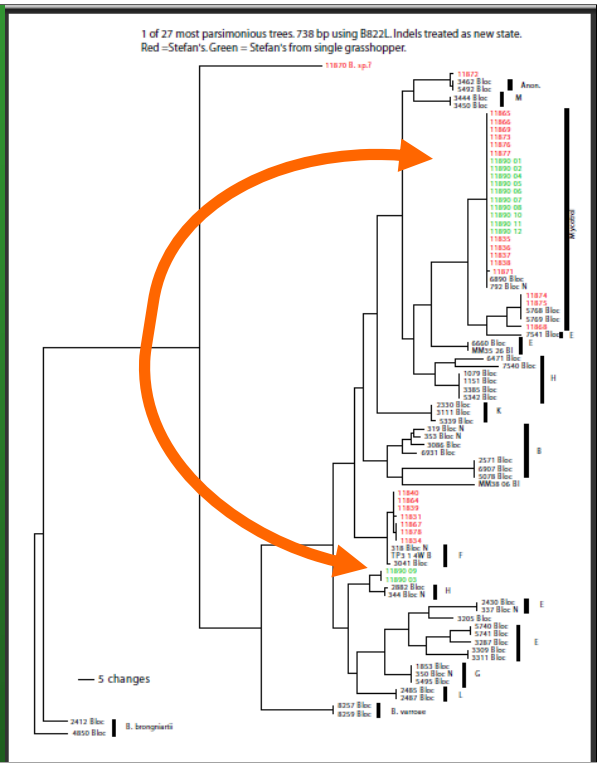
Beauveria can be genetically diverse from one host group (Grasshoppers) in one region (MT-ND)

(AFLP EcoR1-AAC, -AGG Jaronski & Kaufmann, unpubl)



Beauveria can be very diverse even from on insect group (grasshoppers) in one region (eastern Montana-western North Dakota grasslands) The captions in different colors on the right are different locations. The position of the “branch” or fork in the tree indicates similarity with 100% on the far right and 0% on the far left. Most of these Beauveria strains are less than 50% similar even though the originated from just grasshoppers across maybe 100 mile region of grassland!

Genetic Diversity of Beauveria from Grasshoppers in ONE location (and ONE HOST)



And there can be genetic diversity in the Beauveria from ONE LOCATION and ONE HOST! The green numbers indicate 12 cultures derived from single spores from one grasshopper. Two are very different from the other 10. That is, the grasshopper was infected by TWO Beauveria!

Thus, when you see Beauveria on CBB in a coffee farm, you are probably looking at a number of distinct strains.

Host Spectrum of a *Fungus*, *e.g. Beauveria*

1. ***Beauveria*** **as a species attacks all insects, many spiders, some ticks, mites**

2. ***Beauveria*** **isolates have different** ***relative*** **specificities**

"Efficacy" (median lethal dose)

	BB1	BB2	BB3	BB4
Whitefly	1	15	80	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	1000	1000+
Spider Mites	1000+	100	10	1

1-5 = best 7-15 = OK 20-100 = so-so

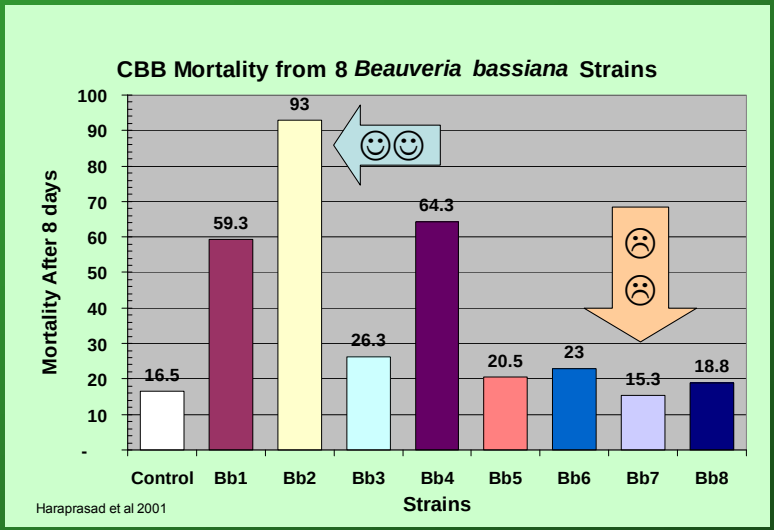
100-500 = not so good >500 terrible

This genetic diversity, genetic differences, is manifested in different physiological attributes, not just DNA sequences.

One attribute is the infectivity of a strain for different insects. While Beauveria as a species can be said to infect a very wide range of insects, each strain can be more specific.

Here's a ficticious example, but based on my experience. The larger the number the less infectious (more spores needed) the strain is for that particular insect. Some strains are generalists, for example BB2, or BB3. Others are a bit less specific, e.g., BB1 which is not infective for bees and spider mites. Some are very specific, such as only spider mites.

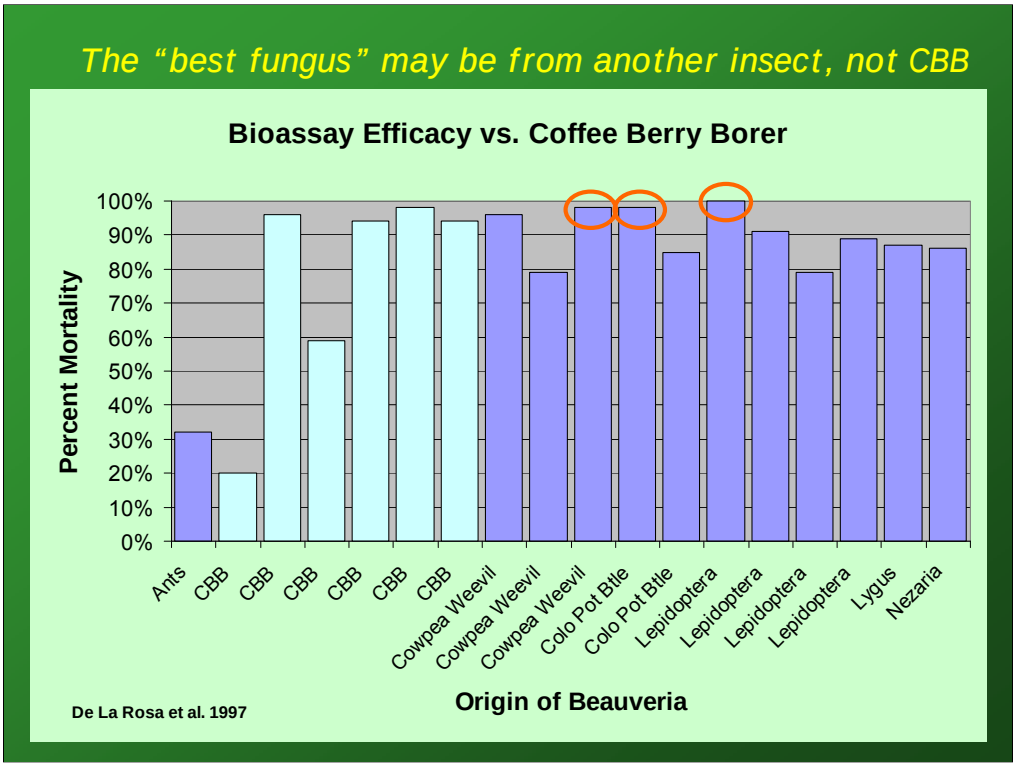
Beauveria strains from CBB can vary widely in efficacy



Immersion assay (1x10^6 conidia / ml for 1 min.)
CBBs were transferred Petri dishes with two healthy coffee beans maintained at ambient temperature (25 C) and 70-80% RH) 8 days.

Beauveria strains from CBB can vary widely in their infectivity and virulence (=effectiveness).

Here's an example from Indian researchers. Bioassay method outlined below the graph). Bb 5-8 were basically non effective even though they were isolated from CBB, while Bb 1 and 4 were much better and Bb2 very effective.



The best fungus strain may NOT be from the target insect, but from a completely unrelated insect.

Here, from a paper by South American researchers, Beauveria from Colorado Potato Beetle, Cowpea Weevil and a caterpillar species were as effective as the best of CBB-derived strains for CBB in a lab bioassay, and some of the CBB strains were terrible.

This makes looking for the "best" fungus difficult.

Beauveria GHA has different infectivities for related species

Efficacy of *Beauveria bassiana* GHA

Target Species	LC50 conidia/mm^2	~ Conidia per Acre	Rank among all other Isolates	~ Rate, qts. Mycotrol O /Acre	Rate for >90% control ?
Fall Armyworm	1213	1.47E+13	31/43	0.74	3.3
Corn Earworm	9	1.09E+11	10/43	0.01	0.1
European Corn Borer	1668	2.03E+13	32/43	1.01	2.6
Diamondback Moth	97	1.18E+12	13/43	0.06	0.4
Beet Armyworm	67	8.14E+11	25/43	0.04	0.1
Wraight et al. Journal of Invertebrate Pathology 103 (2010) 186–199					
Silverleaf Whitefly	270	3.28E+12		0.16	1.6
Wraight et al. Journal of Inverterate Pathology 71, 217–226 (1998)					

What about GHA the Beauveria in Mycotrol O?

Here we have data from work conducted by Steve Wraight and myself with several species of Lepidoptera, and whiteflies. The LC50s here are “spores per square mm of sprayed surface, including the insects on that surface” which LC50s can be roughly related to field rates.

As you can see, GHA is highly virulent for corn earworm but much less so for corn borer and fall armyworm – basically 1000 fold difference.

I have extrapolated these lab data to estimated field rates per acre assuming a leaf area index of 3 (leaf area/acre = 3X acre), a conservative number.

(The next column is the rank of GHA among 43 Beauveria isolates we tested.)

The field rate for theoretical “50% control” by Mycotrol O is next column, and ranges for .01 to 1 quart per acre, based on the lab data.

More realistically, theoretical rates for (theoretical) 90% control is in the last column, in white. These numbers are based on the “dose regression slope” in the bioassays – these slopes, indicating how much more fungus is needed for incrementally greater kill of the insect, are generally very low, meaning a lot more fungus is needed to go from say 50 to 90% kill, than with a chemical insecticide. So the rates of Mycotrol O for 90% kill range from .1 to 3 quarts per acre.

Of course these rates ignore a lot of factors in the Real World that would probably increase the needed rates even more.

Nevertheless, you can see how GHA varies in its efficacy against different, but related insect species

Effect of Bb GHA Formulation on Efficacy

(and effect of stadium on susceptibility)

	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

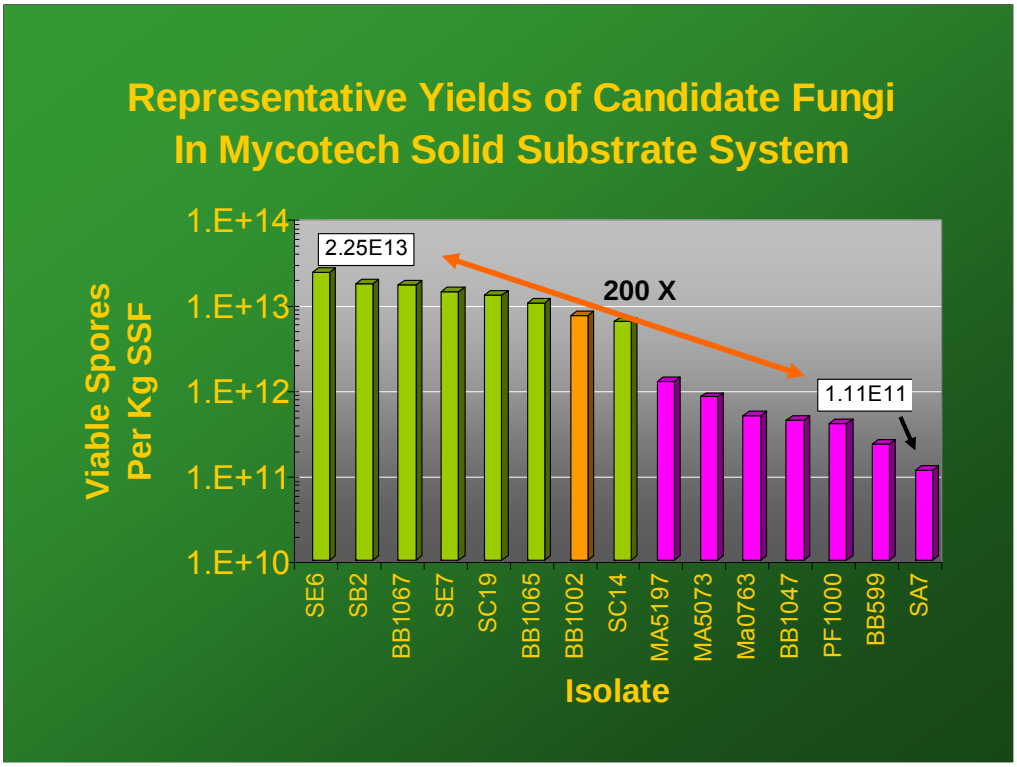
B. Murphy, unpublished data

And the **stage of insect** and **formulation** can affect performance of a fungus, here, Beauveria GHA, against an insect. These data were generated by Brook Murphy in the 1990s using sprayed miniature roses in the greenhouse – so the data are somewhat realistic. He used 0.5 qt of ES or .5 lb WP /100 gallons of spray, applied to the miniature roses to just short of runoff.

Thrips: Note the difference in efficacy of GHA for adult and larval thrips: The ES formulation has a real difference; the WP formulation does not! Why?

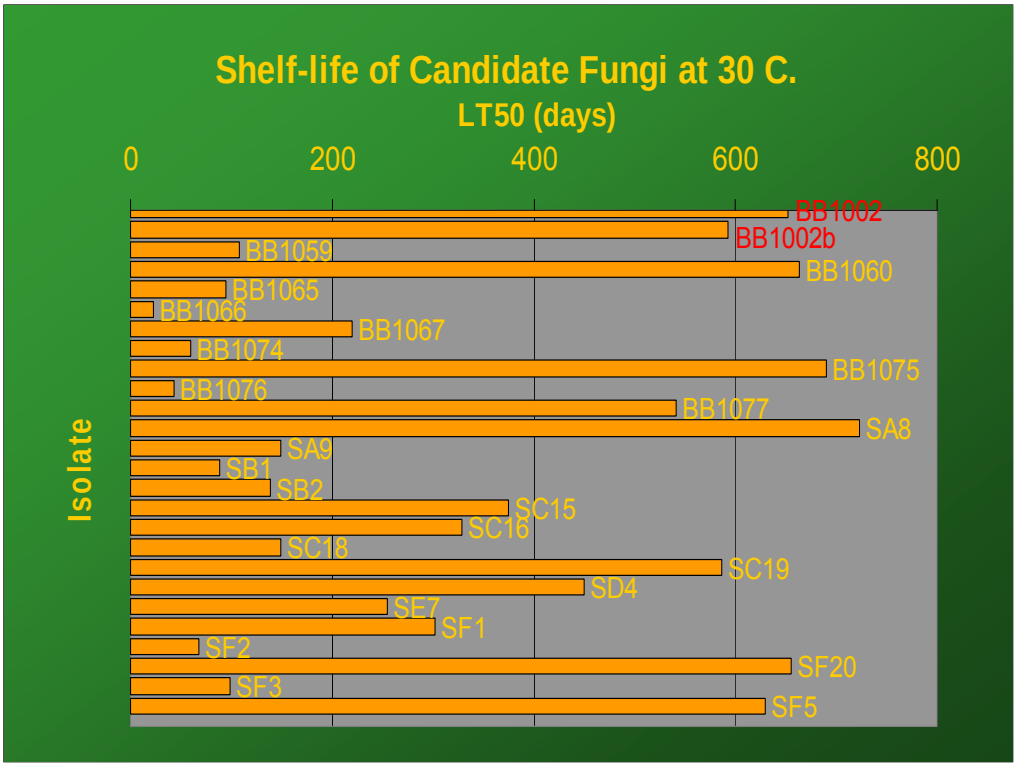
Reasons have to do with the behavior of the insect where the adult is very cryptic, living inside opening and open flowers. The ES did not penetrate well into these cryptic habitats, but the WP, using a really good spreading agent, Silwet L77, did. More about this spreader later on.

Cotton Aphids: really big difference between aphid adults and nymphs in susceptibility to the ES formulation. And also a difference with the WP formulation of GHA. And the WP is terrible for both. Why the difference with the ES and WP? Well, immature aphids molt very frequently, even every 24 hours (when did I mention 24 hours earlier???) Thus nymphs can easily shed germinating, penetrating spores when they molt so often. Adults however, don't molt. Why the difference between ES and WP with adult aphids?



Fungal isolates can vary greatly in their ability to produce spores – another genetic trait. Look at the range of yields of these Beauveria strains grown under the same conditions. BB 1002 is Laverlam's strain GHA, in Mycotrol O.

Critical cutoff for commercially feasible mass production is around 1x1013 spores per Kg of grain substrate (green bars). Less than that makes the fungus more and more expensive to produce (magenta bars). And it seems often in my experience that virulence for insects and spore production are inversely related – the best strains are poor spore producers.



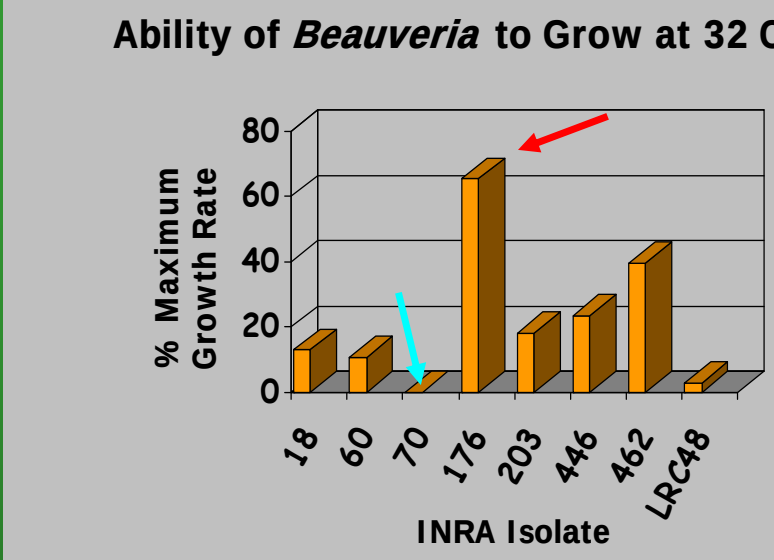
Shelf life of different isolates can vary greatly also. It seems that shelf life (persistence of spore viability over time) is at least partly due to genetics. Here are data on the half life (LT50) of a number of Beauveria isolates grown up and harvested under identical conditions and stored as conidial powders in Nalgene vials at 30 C. This was part of a program to develop a Beauveria for control of larval fleas in backyards, etc.

"BB1002" (red) is two lots of the GHA strain of Mycotech.

Note the variability. Some isolates are as good as GHA (which was chosen partly for its excellent shelf life) – BB1075, 1060, S48 Others – BB1059, 1065, 1066 have a very short persistence of the spore viability

So here is another aspect in which Beauveria strains can vary, based on their genetics.

Strains Vary in Tolerance to Temperature

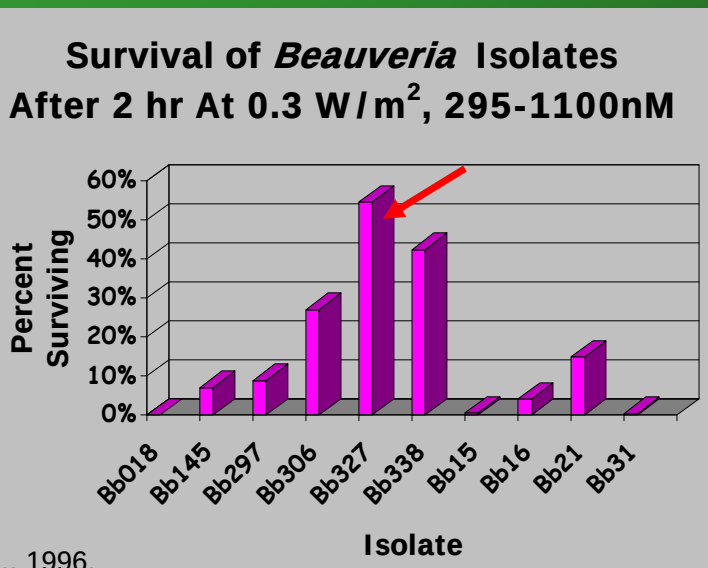


Fargues et al., 1997. Mycologia 89:383-392.

Fungus (here Beauveria) strains can also vary in their apbility to grow at different temperatures, esp above 30 C which can be very important for fiedl efficacy.

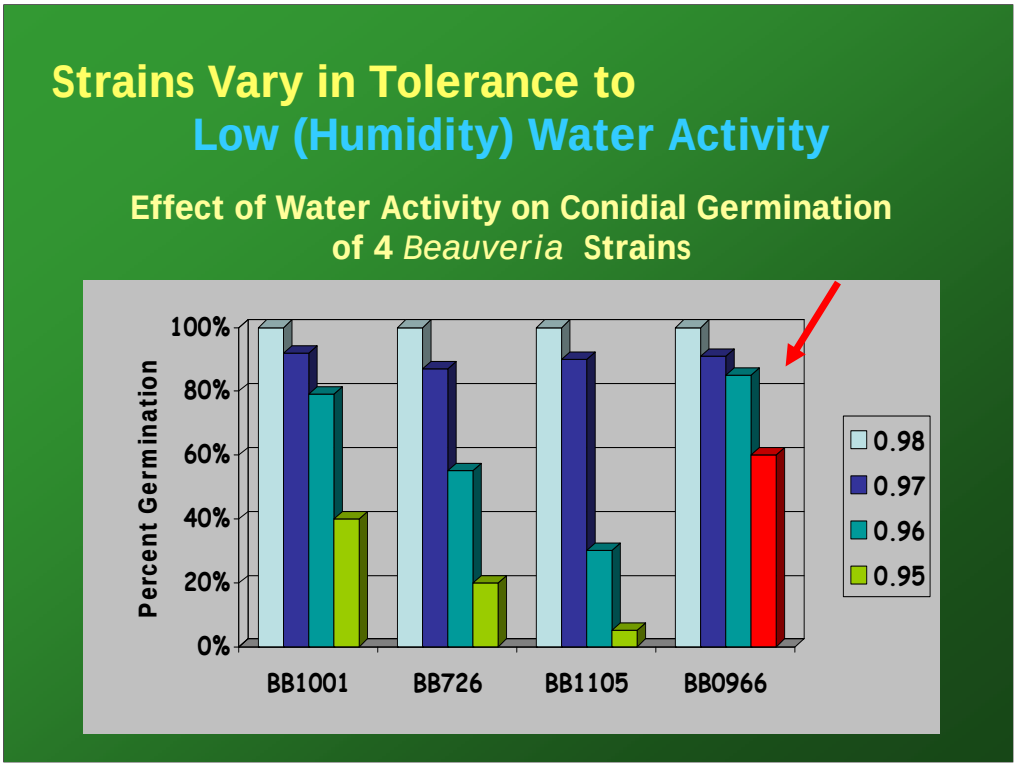
INRA isolate 176 might be ideal for warmer situation, while 70 grew better at lower temperatures than the others, thus cold be ideal for cooler uses, all other facets and factors ignored …

Strains Vary in Tolerance to Ultraviolet Radiation

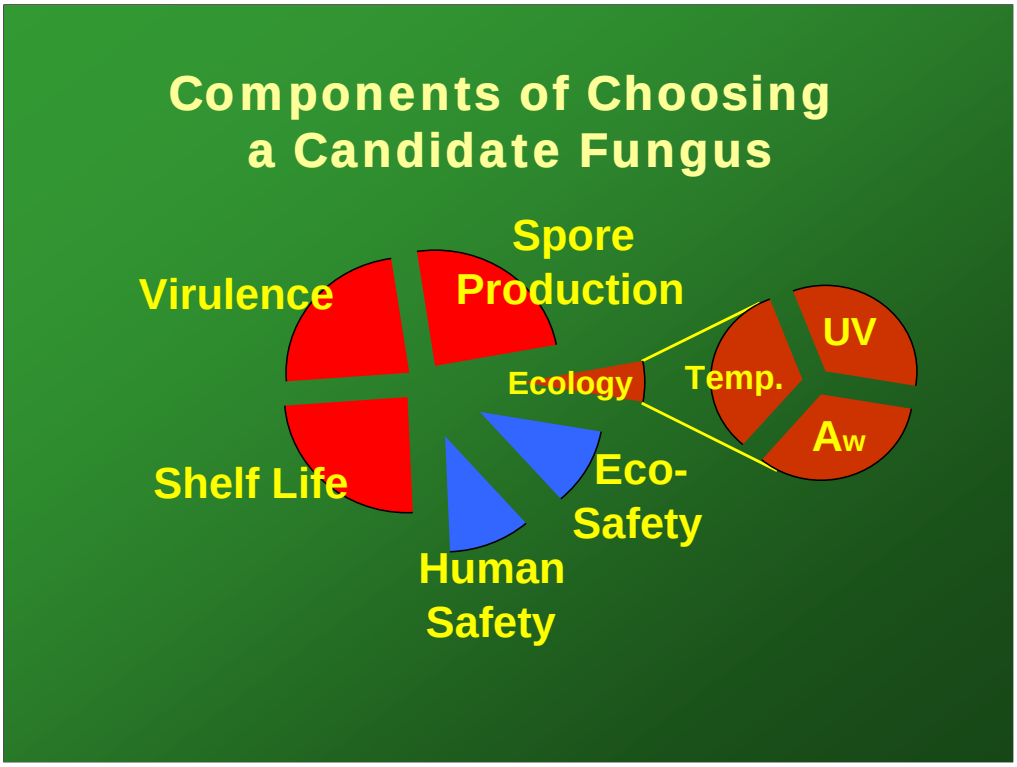


Fargues et al., 1996.
Mycopathologia 135:171-181

Fargues and associates demonstrated that Beauveria isolates can vary in their tolerance to UV. Obviously BB327, and 338 might be better suited for foliar use, IF their virulence, spore production, shelf life, safety characteristics were OK.



Isolates can also show differences in threshold water activity for germination. Here, BB0966 germinates better at a “Water activity” (Aw) of 0.95 (= 95% humidity), a substantially drier condition than can the other isolates. (1.00 = 100% RH; .95 = 95% RH). A fungus that can germinate at a lower humidity may have an advantage over other strains, depending on the ecology of the target insect.



Choosing a commercial microbe has a number of components – a mosaic of criteria, if you will, not just virulence.

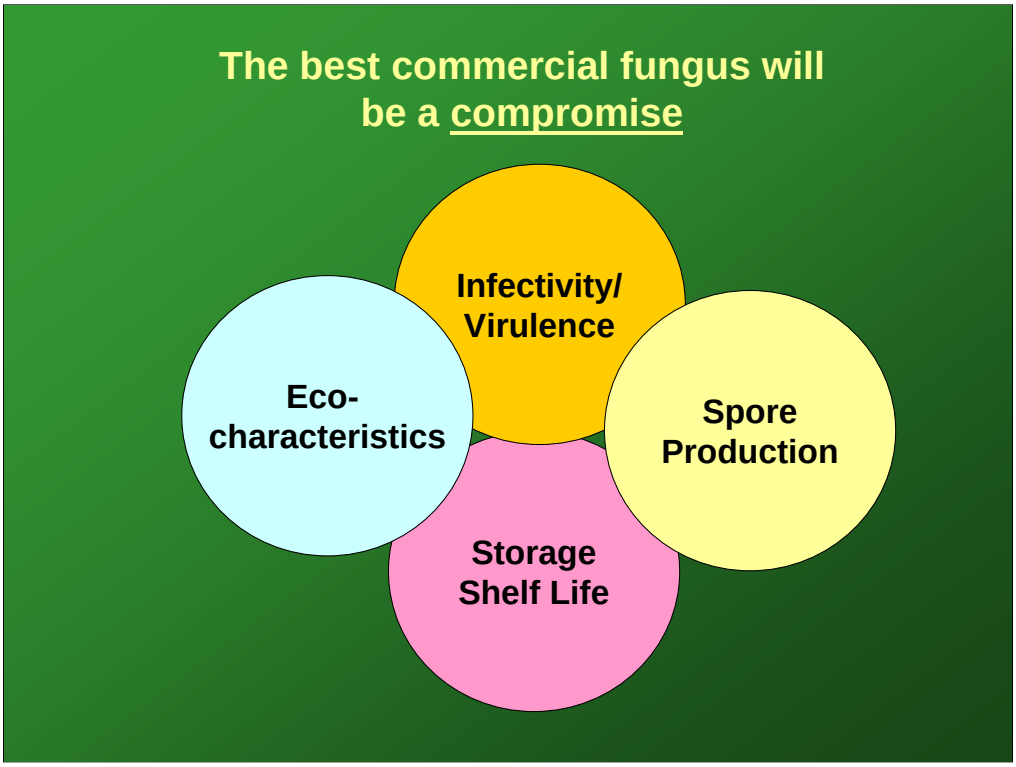
Commercially economic spore production is as important.

Shelf life is also important. If one can't keep a microbial alive "on the shelf" for at least a year at room temperature, then it will be difficult/expensive to market.

Human safety of a microbial is paramount (and Beauveria, Metarhizium and related fungi are safe – not infectious for healthy humans and vertebrate animals).

EPA assumes some degree of non target adverse effects, but as long as the effects of the microbial are *less than* the currently registered pesticides, adverse effects are not fatal to registration.

By ecology I mean those attributes of a candidate that fit it for the environment of its intended use: temperature tolerances,resistance to UV irradiation, critical moisture (Aw or water activity) for spore germination and growth.



Thus selection of a fungus, like Beauveria GHA by Mycotech Corp. is often a compromise – the strain may not be the BEST killer, but “good enough” given degree of mass production (unit of fermentation (=$$) per acre of use, and have good shelf life. And also work under the intended ecological conditions.

What about *Beauveria* GHA?

- In general, a pretty wide target spectrum with reasonable efficacy for key target pests
- Excellent spore production
- Excellent genetic stability
- Excellent shelf life even without formulation
- Favorable safety testing data

What about Beauveria GHA, the active ingredient in Mycotrol O?

Life gets complicated outside of the scientist's laboratory ...

Beauveria GHA lab efficacy against grasshoppers

Efficacy of Bb GHA with Grasshopper Species	LD_{50} conidia /insect
Melanoplus sanguinipes	49,700
M. differentialis	1,430,000
M. bivittatus	320,000
M. packardii	640,000
M. femurrubrum	760,000
Phoetaliodes nebracensis	37,800
Schistocerca americana **N5**	376,000
Anabrus simplex N4	90,000
A. simplex **adults**	300,000

Jaronski unpublished data

In lab bioassay Beauveria GHA has the efficacies shown here -- LD50's (not LC50 because with grasshoppers I can apply discrete numbers of spores to each insect) range from ~38,000 spores per grasshopper to 1.4 million spores.

The last two lines are for the Mormon cricket, which is actually a katydid, not cricket or grasshopper, and can be a real nuisance in ID, NV, WY. (Note the difference between nymph and adult with the nymph being much more susceptible.)

Beauveria GHA lab efficacy against grasshoppers

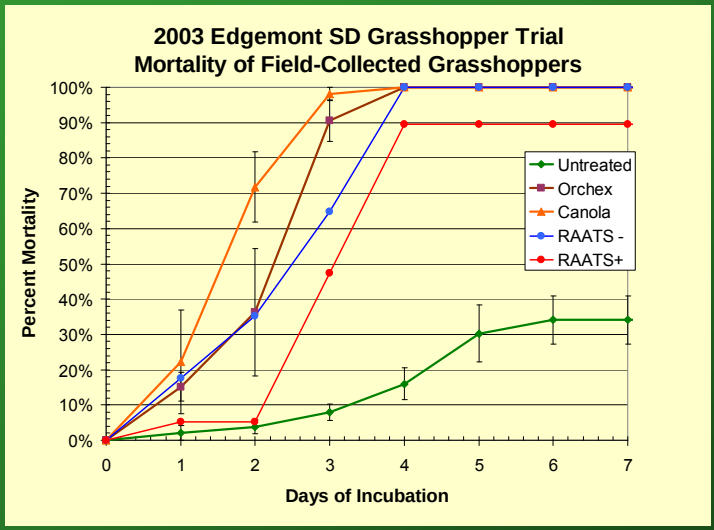
Efficacy of Bb GHA with Grasshopper Species	LD_{50} conidia /insect	~ Conidia per Acre	~ Rate, qts. Mycotrol O /Acre	Rate for >90% control ?
Melanoplus sanguinipes	49,700	1.37E+12	0.1	0.7
M. differentialis	1,430,000	1.63E+13	0.8	8.1
M. bivittatus	320,000	3.64E+12	0.2	1.8
M. packardii	640,000	1.76E+13	0.9	8.8
M. femurrubrum	760,000	2.09E+13	1.0	10.5
Phoetaliodes nebracensis	37,800	1.04E+12	0.05	0.5
Schistocerca americana **N5**	376,000	4.28E+12	0.2	2.1
Anabrus simplex N4	90,000	2.48E+12	0.1	1.2
A. simplex **adults**	300,000	8.27E+12	0.4	4.1

Jaronski unpublished data

I've taken the lab data and extrapolated all the way to a theoretical rate of Mycotrol O per acre for 90% control of each grasshopper. Rates range from .5 to 11 quarts per acre (applied broadcast to open rangeland).

What happens under real world conditions?

Replicated 40-acre rangeland plots aerially treated with 1x10^{13} conidia / acre *Beauveria bassiana GHA*. Edgemont, South Dakota, 2003.

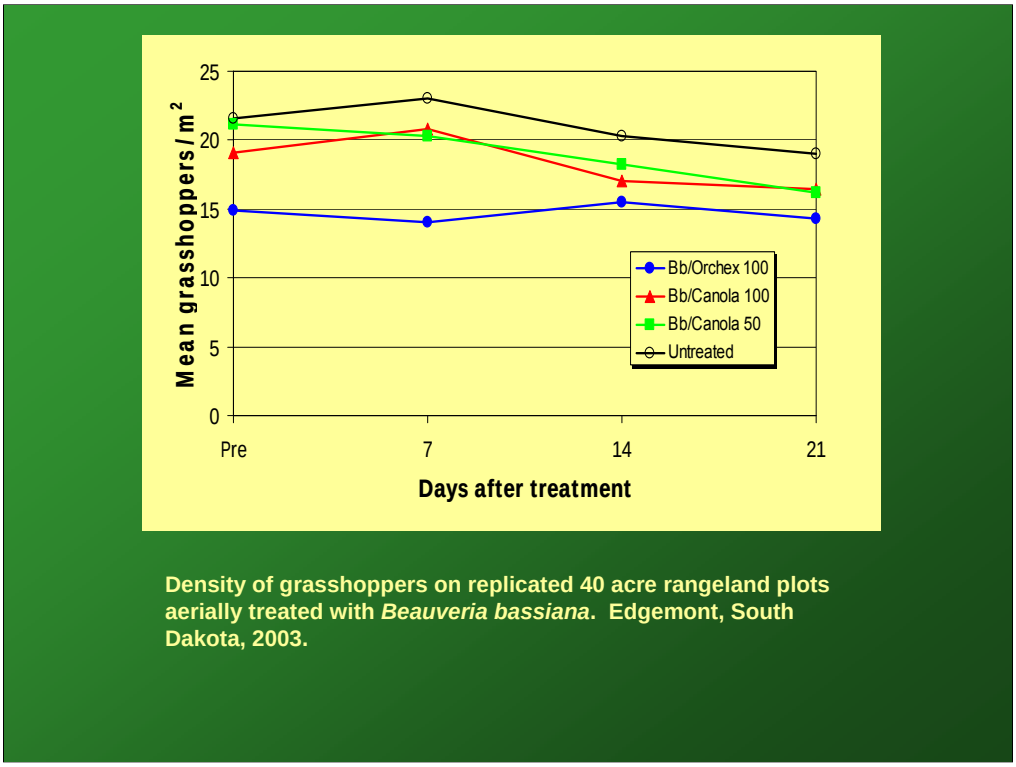


Jaronski & Foster, unpublished

These are data from a field trial we conducted in 2003, using equivalent of 0.5 quarts Mycotrol per acre.

This graph represents mortality from fungus infection among grasshoppers collected from the treated fields a few hours after the fungus was applied and incubated in my motel room (don't worry about the different treatments; they're just some fine details). In 3-4 days most or all are dead from fungus infection.

Great!



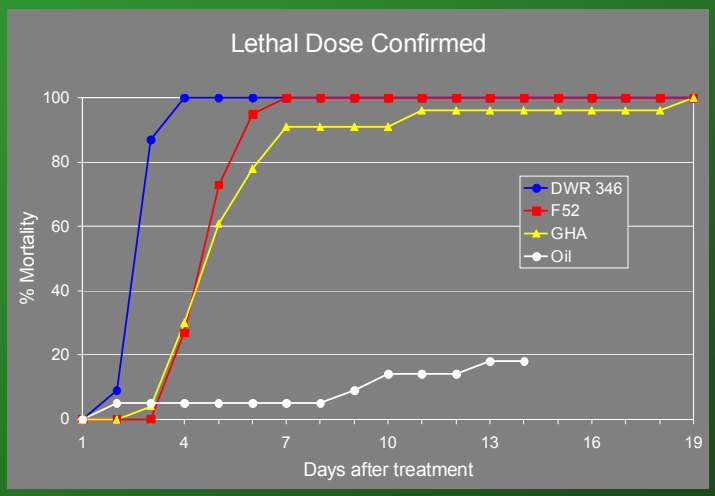
Density of grasshoppers on replicated 40 acre rangeland plots aerially treated with *Beauveria bassiana*. Edgemont, South Dakota, 2003.

But this is what we saw in the field populations – grasshoppers left out, doing their thing in nature. There were no population reductions, no control by the fungus, no body really dying off.

What is going on?

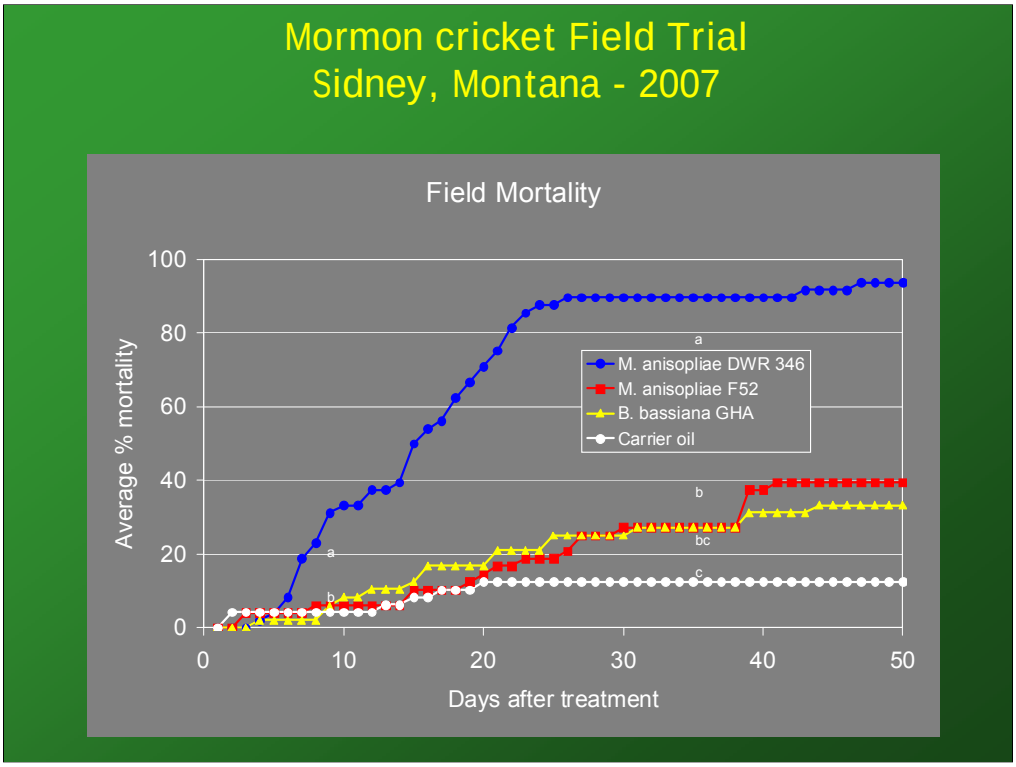
Mormon cricket Field Trial Sidney, MT 2007

1.5 qts. (1.5×10^{13} conidia) / acre ULV oil spray
Nymphs collected 3 days after treatment; incubated in lab at 28° C

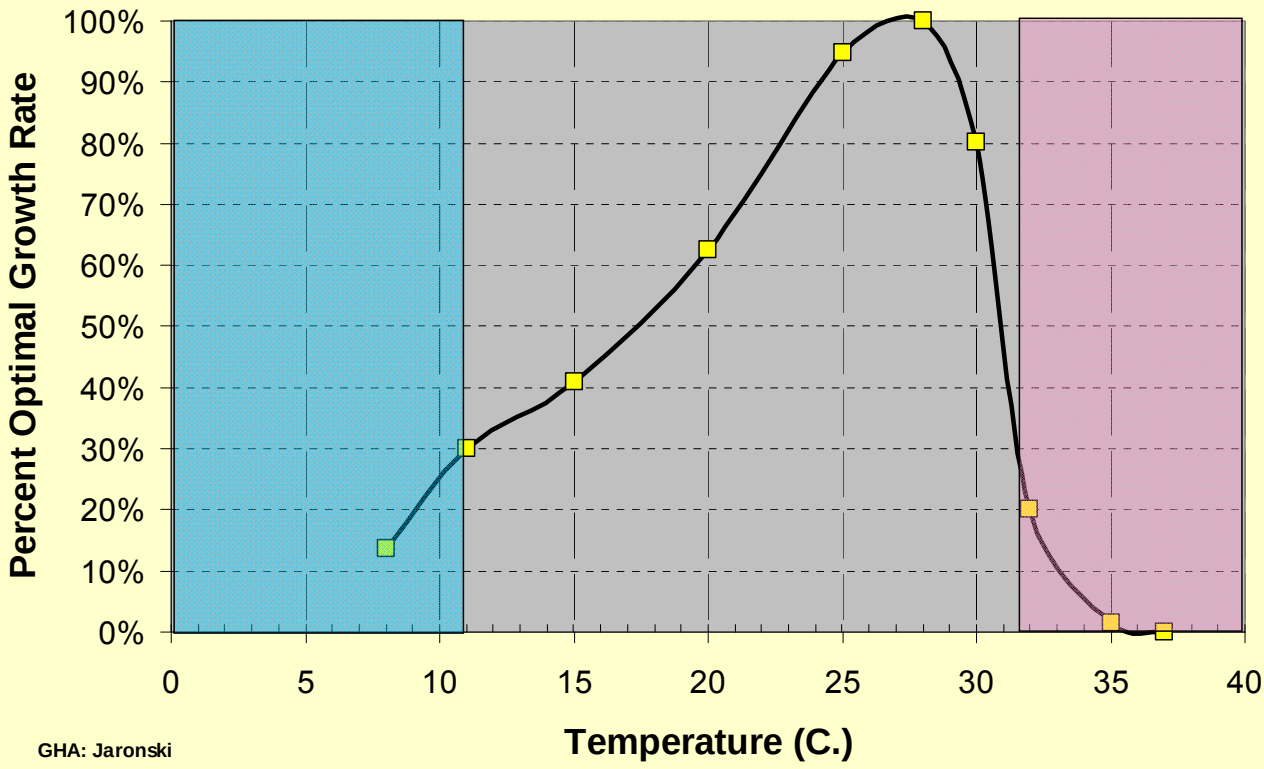


Foster and Jaronski. In press

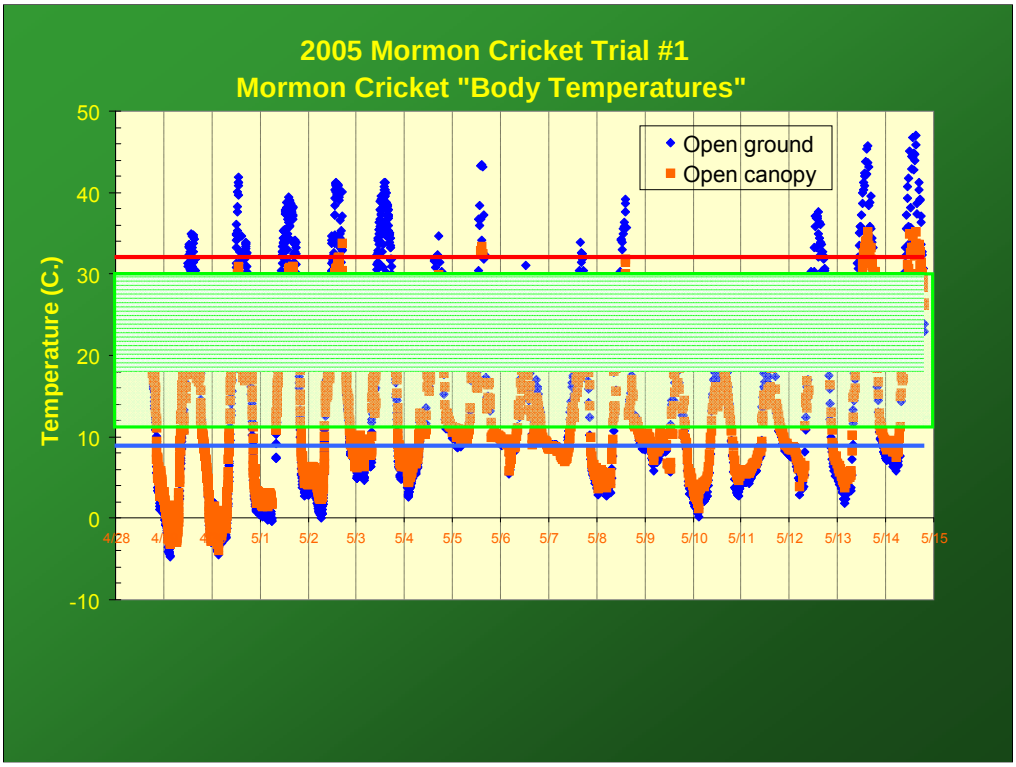
Here's a trial with Mormon crickets, done in 2007. Insects brought into the lab two days after exposure to sprays and spray residues of Beauveria GHA (as well as two other fungi), and incubated at a temperature ideal for the fungus, died off very rapidly. Beauveria GHA is the yellow line .



And this is what happened in the field. Neither Beauveria GHA nor Metarhizium F52 had much affect on the Mormon cricket populations (the third fungus DWR346 did, but still took 20+ days to kill the insect, versus 3-4 days in the lab...



Here are thermal tolerances for Beauveria GHA, drawn from the literature and my own studies. Best growth of GHA is at 23-30 C. At 11 C growth is only 30% of fastest rate; at 32 C the same. Note also how growth falls off quickly as the temperature increases above 28.

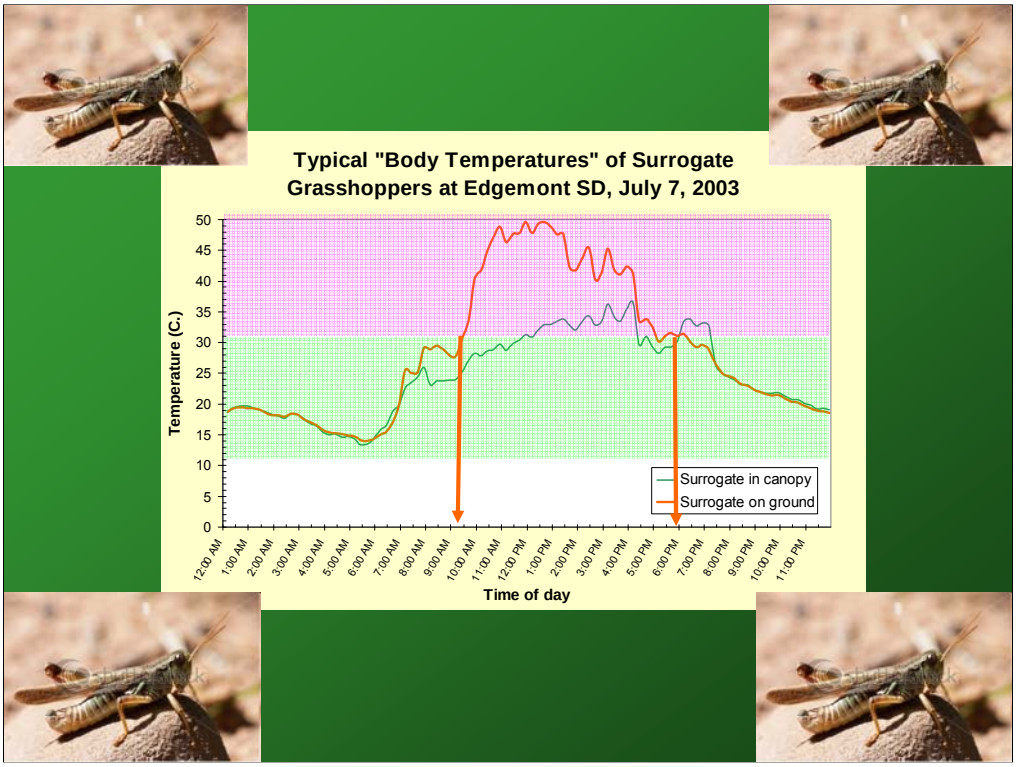


Here's The Rest of the Story:

Both grasshoppers and Mormon crickets actively thermoregulate by basking in the sun. Here are body temperatures of Mormon crickets on the ground and in plant canopy recorded continuously for a number of days during a field trial. The green zone represents temperatures really good for Beauveria GHA growth – 11-30 C. The red bar is the upper thermal limit for fungus growth and the blue bar the lower limit (and yes we did get -2 C on April 29 and 30, 2005).

Note how many hours of each days insect body temperatures are outside the green zone.

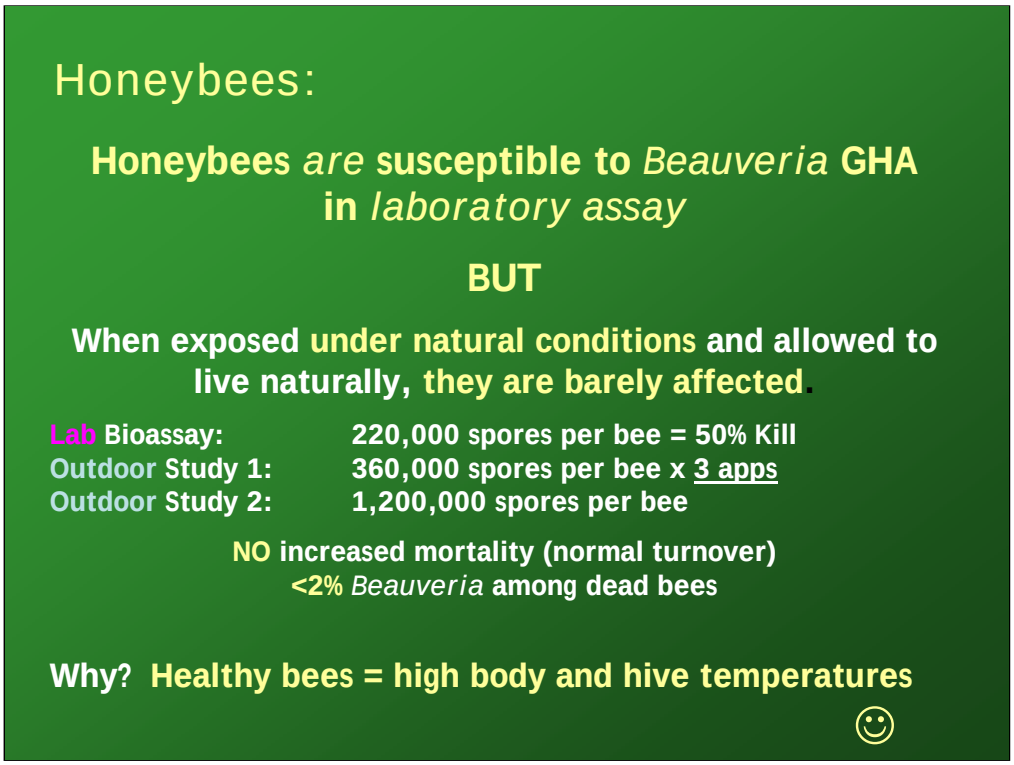
Such body temperatures greatly limit the number of hours each day that the fungus can grow and really set infections back.



Grasshoppers similarly thermoregulate, even sitting on bare ground and rocks to absorb the warmth from the sun. Here are data from one day in 2003 during a grasshopper field trial. By 9 AM the grasshoppers are heating their bodies above the temperature for Beauveria growth, and don't "cool down" until 6 PM. (Grasshoppers don't really heat themselves all the way to 50C – this is an artifact of fixed sensors rather than mobile grasshoppers. But the hours per day are accurate.)

In addition when they sense infection by a pathogen, increase that basking to heat themselves up to 39-41 C, becoming in a sense, 6-legged saunas. This phenomenon is called "behavioral fever" and can successfully stave off death from fungus infection, as I showed you previously.

The same ecological and behavioral barriers can protect non target insects too.



In 1995-1997 I and colleagues at Mycotech conducted a series of honeybee tests with Beauveria GHA for EPA.

In the lab an average of 220,000 spores/bee killed 50% of them in 5-6 days.

We then simulated exposure of bees to GHA sprays applied by a farmer. In the first test all the workers from replicate colonies were removed, anesthetized with cold, and sprayed GHA at the equivalent of 1 quart per acre of Mycotrol, three time sat 5 days intervals (applications like a melon farmer would do). The average dose was 360,000 spores per bee at each spray. In the second test, at EPA's request, we applied 5X the field rate, delivering 1.2 million spores per bee.

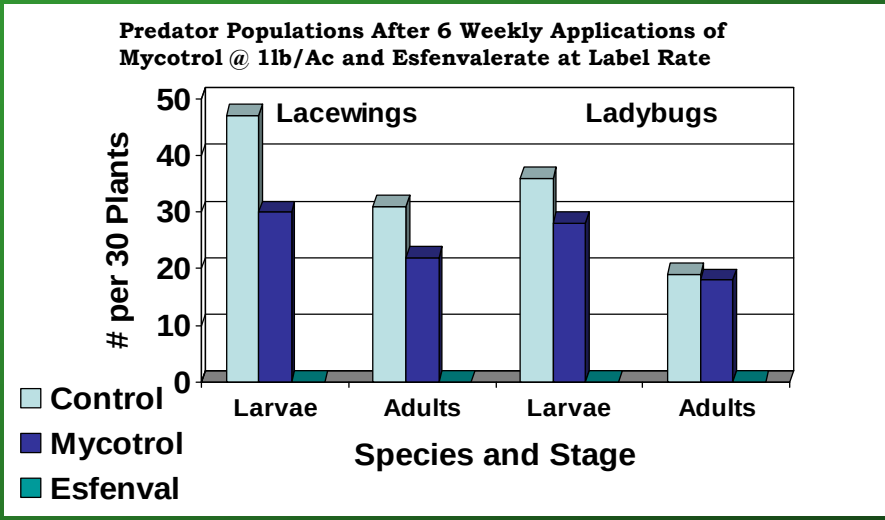
The result? No increased mortality beyond the normal turnover of worker bees; no infections among the larvae. Less than 2% of the dead bees had Beauveria infections.

Why? "A happy bee is a healthy bee" Bees allowed to live normally, in contact with their queen, have body temperatures and hive temperatures above 32 C. regardless of the weather outside.

Bees have even been used by Canadian researchers to carry Beauveria spores to canola for the control of Lygus bugs!

Predators:

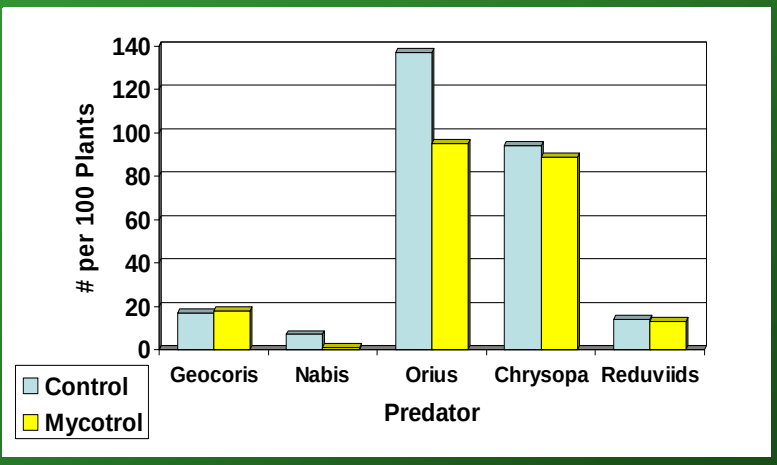
***Beauveria* GHA has very little to no effect on predators in the field.**



Similarly many predators, which are quite susceptible to a fungus like Beauveria in the lab, have an ecology and behavior that at least partially protects them from the fungus in nature. Here are results of a field trial in cotton, involving 6 weekly applications of Beauveria GHA in a wettable powder formulation or Esfenvalerate, a synthetic pyrethroid. While esfenvalerate wiped out both predators, the fungus had only slight impact (numbers for adult lacewings and both ladybug larvae and adults were not significantly different from the control. In the lab both insects are very susceptible to the fungus.

Predators: *Beauveria* GHA has very little to no effect on predators in the field.

Effect of Mycotrol 22WP (2 lb, $4x10^{13}$ / Ac)
7 days after application to cotton (Phoenix 1996)



Jaronski et al 1998 Brighton Crop Protection

Here's another, multi-acre study I conducted in Arizona cotton back in '96, where I applied 2 lb of Mycotrol WP per acre. Only Orius was significantly affected by the fungus but numbers decreased less than 50%

Parasitoids:

Beauveria GHA has no impact on *Eretmocerus* in the field

- **60,000 *Eretmocerus* released then Bb GHA applied at $2x10^{13}$ / acre 3X, weekly intervals**

Rate of Parasitism Unaffected

Treatment	Control	Beauveria
Day 2 P.T.	39%	34%
Day 18 P.T.	42%	31%

Parasite population (parasitized nymphs/cm^2 leaf):
0.9 vs. 0.8 on Day 2 p.t. (nsd)
1.2 vs 2.4 on Day 18 p.t. (nsd)

Overall reduction of WF popln (nymphs/cm^2): 71%

Jaronski, Simmons & Hoelmer, unpublished

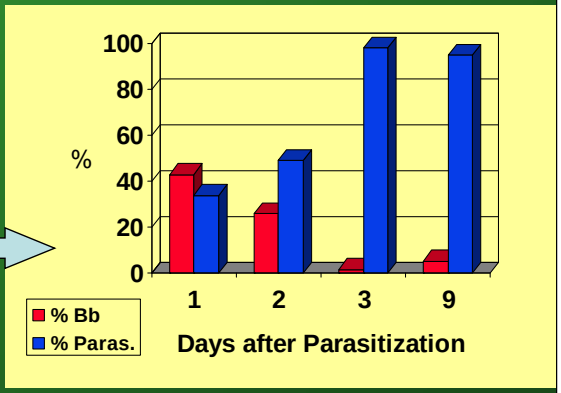
It can be the same situation for parasitoids. Here I and my coworkers released a whitefly parasitoid into a patch of organic cantaloupe, allowed the Eretmocerus to establish, then applied Beauveria three time at weekly intervals. Bottom line: RATES of parasitism were unaffected and the number of parasitized whitefly nymphs were not significantly different between the Beauveria and Control treatments. (Overall reduction in the number of whitefly nymphs was 71%) Oh yes, the Eretmocerus is very susceptible to GHA in the lab...

Brook Murphy has also observed similar results for aphid parasitizing wasps in Easter Lillies.

Parasitoids: Why little effect?

Key fecundity is NOT affected

- **Female wasps can detect infected nymphs!**
- **Parasitized nymphs lose susceptibility to fungus!**

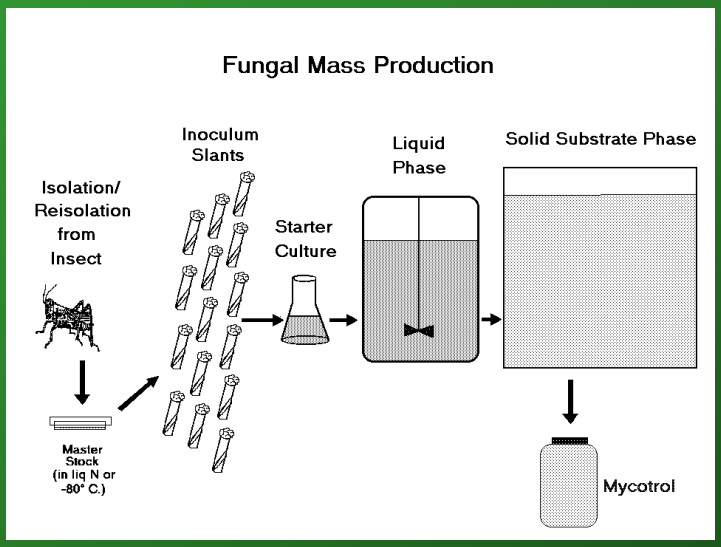


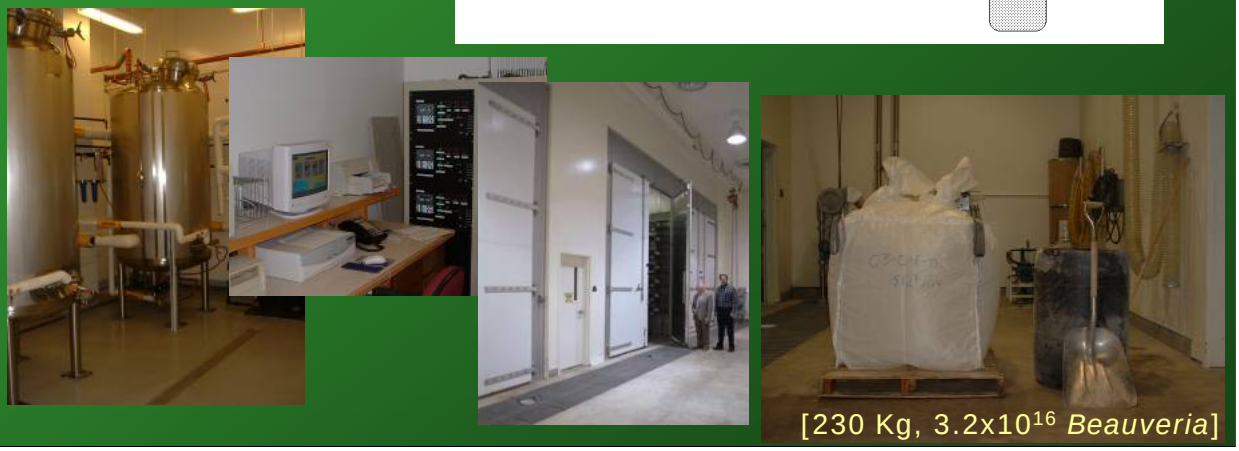
(Jones & Poprawski, 1996)

The key aspect is that parasitoid fecundity is not affected

1. We know that this and other wasps can detect fungus-infected nymphs and will skip them, placing eggs in only healthy insects
2. Jones and Poprawski also observed that parasitized whitefly nymphs lose their susceptibility to the fungus after the second day of parasitization! Evidently the wasp larva releases some sort of chemical that inhibits infection.

How are these fungi produced?





This is a schematic of the commercial scale production such as practiced by Laverlam.

The fungus is isolated from an insect to make a “mother culture” (the crown jewels) which is stored in replicate at -80C or in liquid nitrogen

This in turn is used to prepare many slants of inoculum. At Mycotech we would prepare on the order of 200-300 slants, which would last 1-2 years.

A slant is then used to inoculate 1-2 L liquid culture, which in turn is used to inoculate 1500 Liters. The blastospore culture is then used to inoculate up to 10,000 Kg of sterilized solid substrate, usually a grain.

Fermentation of the Beauveria is computer controlled to very fine tolerances of temeprature and humidity, keeping environment optimal for the fungus.

After 1 week the whole culture is dried and the conidia harvested by physical separation from the dry culture and purified from debris by mechanical classification, formulated as the ES or WP, and packaged.

Note the size of the fermentation chambers in the center photo....

Each fermenter can produce 230 Kg of pure Beauveria spores

The challenge is to turn these conidia into something the farmer can easily use
That's the formulation challenge

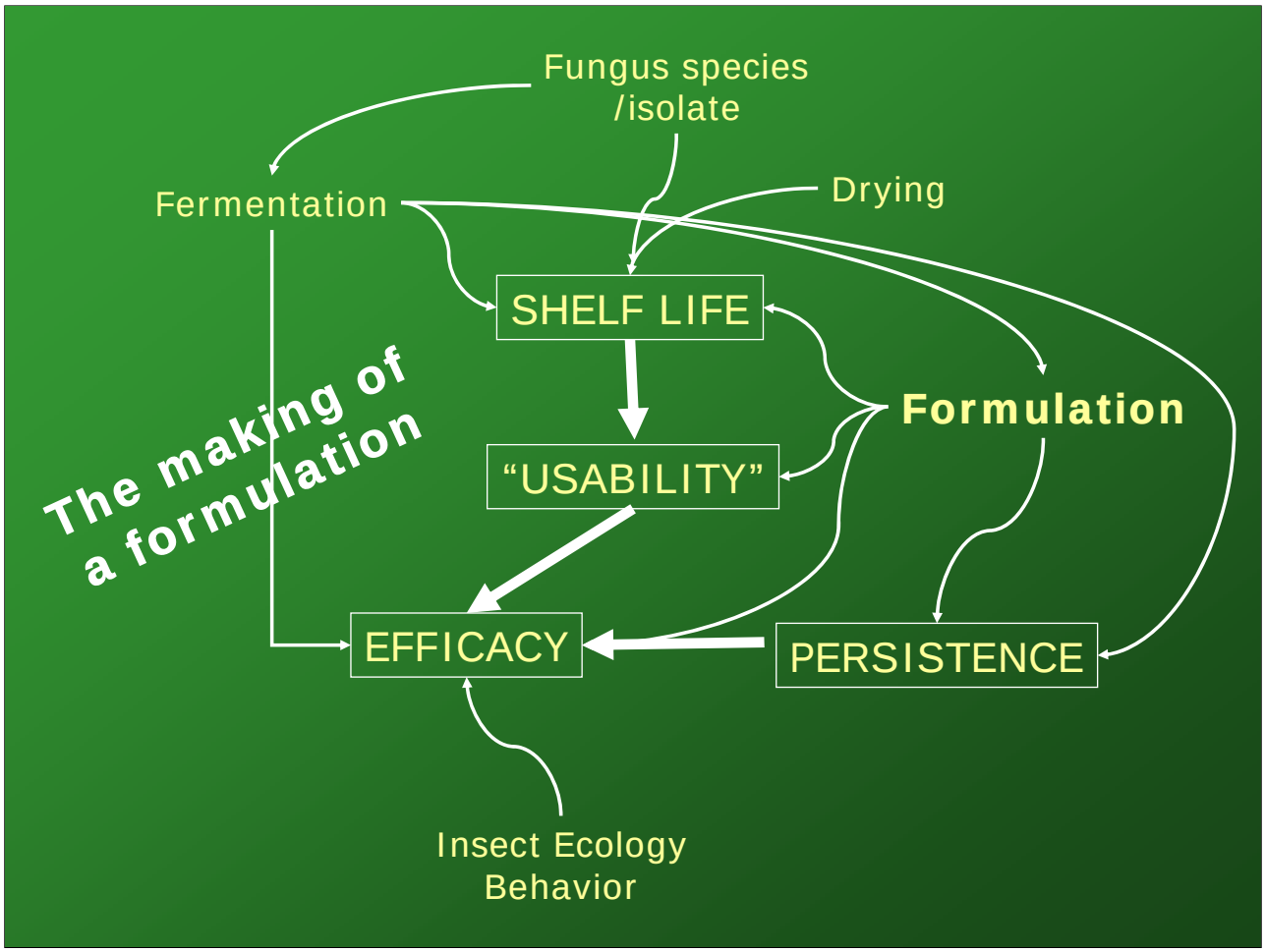
Key mycoinsecticide objectives

- "Good" shelf life
- Easy application
- Good Efficacy

- Safety
- Cost Effective

The keys to a good mycoinsecticide formulation are …

Shelf life typically defined as "acceptable" loss of conidial viability after 1 year at room temperature (20-28C)



But the situation is a complex one, with a number of factors interacting to affect shelf life, usability, persistence, efficacy.

Mycoinsecticide Formulation Requirements:

Extremely hydrophobic conidia need to be suspended in water for many uses

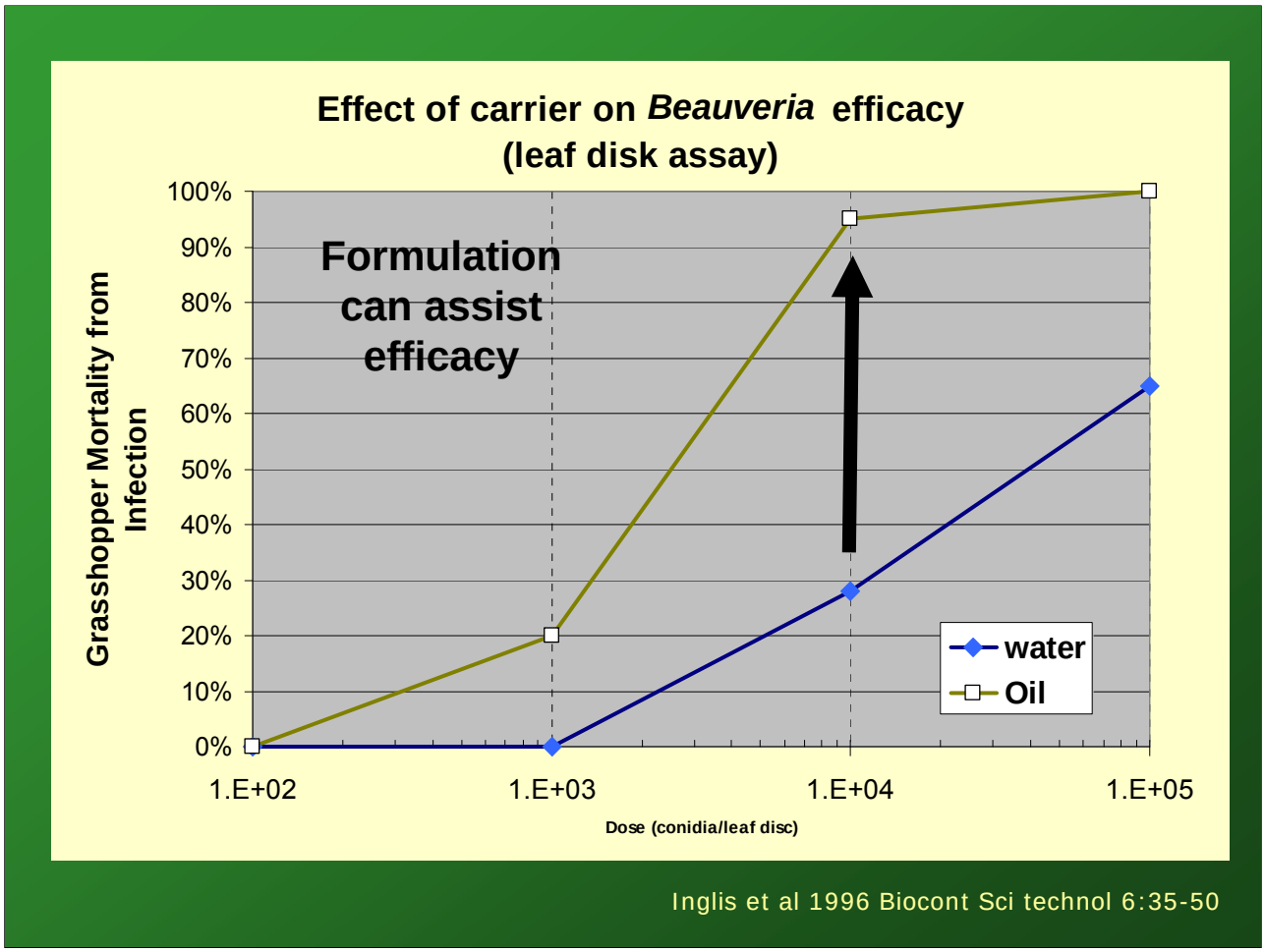
Beauveria tgai
in 0.1%
Tween 80

After 5 hours …

Those of you who have worked with conidial powders of Beauveria or Metarhizium will be familiar with the difficulty of suspending the very hydrophobic conidia in water even with many dilute nonionic wetting agents.

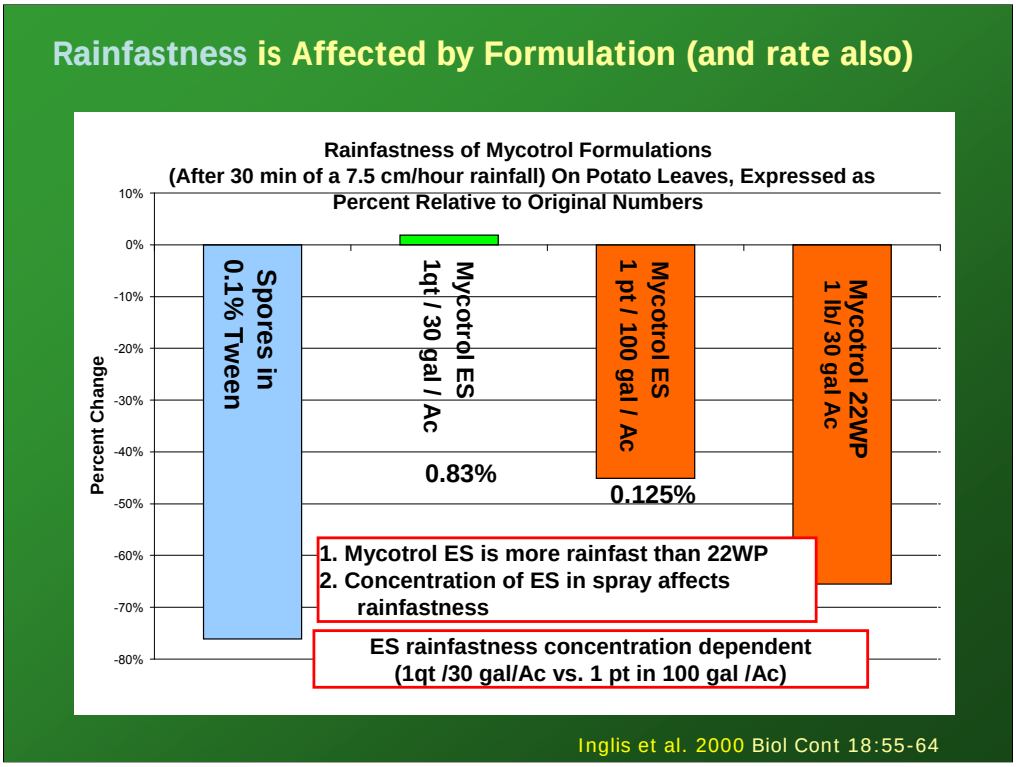
[Video clip] On left you can see how the conidia “refuse” to go into suspension… I takes very vigorous energy input (agitation) to suspend dry conidia.

On the right is the same beaker after 5 hours. Notice how the conidia have remained unwetted and form distinct surface scum.



There are several demonstrations that oil carrier can enhance infection by these fungi. Here, from a paper by Inglis et al,, Beauveria infectivity was enhanced by oil carrier in comparison with water as carrier.

This is because the insect cuticle is water repellent and lipid loving. Thus when an oil droplet with spores lands on insect cuticle it spreads and “glues” the spores to the insect.



Formulations can also confer rainfastness.

Spores in a nonionic wetting agent, or the WP formulation are not very rainfast on plants.

Neither is the ES (and O) formulation, when too diluted in water. 1 pt of formulation in 100 gal water was not rainfast, but the same formulation at 1 pt in 30 gal was.

A digression:

Registration of microbial pest control agents is necessary

USEPA,
then individual states.

Why?

Federal Insecticide Fungicide Rodenticide Act 7 USC 136 "FIFRA"

- Prohibits
  Sale or Distribution
  of any substance for
  preventing, repelling,
  destroying, mitigating
  a pest
- *Includes* viruses, bacteria, fungi, protozoa

Note that the DISTRIBUTION as well as sale is prohibited, although theoretically one can "grow their own" for personal use without registration. But don't give it to your neighbor!

Registration Data Requirements

- Product (organism) characterization
  - ➔Classical and molecular identification, genetic stability
  - ➔Id of unintended ingredients & toxins, "5-lot analysis"
  - ➔Physical/chemical properties, shelf life, persistence
  - ➔Analytical methods
- "Tier 1" safety testing
  - ➔Mammalian Infectivity/pathogenicity/toxicity
  - ➔Birds and Freshwater Fish
  - ➔Freshwater/Marine Aquatic Invertebrates
  - ➔Honeybees and Non-target Insects
  - ➔Non-target plants (phytotoxicity of formulations)

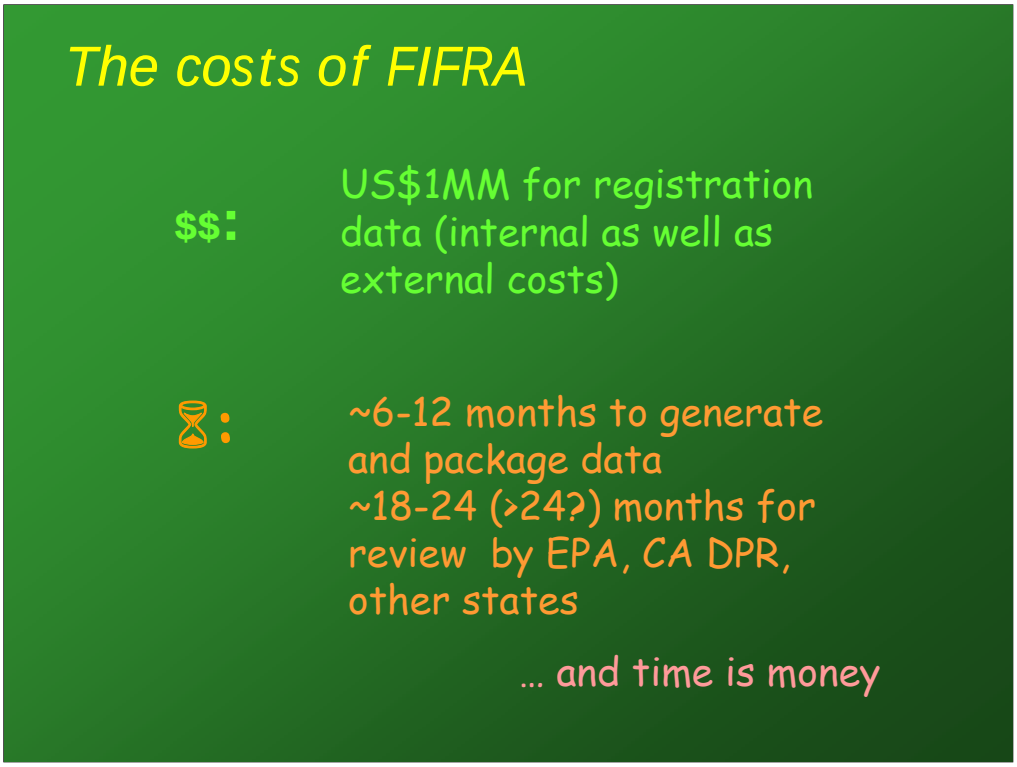
All or most studies under **Good Laboratory Practice (=$$$)**

You may be familiar with some of the registration requirements, esp. the safety testing, but there is a lot more data to be generated, such as all the characterization tests listed here.

All or most under Good Laboratory Practices, which drives up the costs of these studies

It's a lot of work: the data package for Beauveria GHA was 1500 pages:

What also complicates matters is that EPA considers **each strain or isolate of microorganism a unique entity**, requiring data specific to it. Bridging data from other strains of the same species is not allowed. Thus we have separate registrations for GHA and for ATCC 70147 – the fungus in Naturalis, for Metarhizium anisopliae ESC 1 and for F52 (Earth Bioscience's newly registered fungus).



The $1MM is a conservative estimate, could be as high as 1.5MM

Example: Cost of 1993-97 tests:

$24,000 for ladybug study

$15,000 for earthworm test

$200-240,000 for acute mammalian toxicology tests

Back to *Beauveria* …

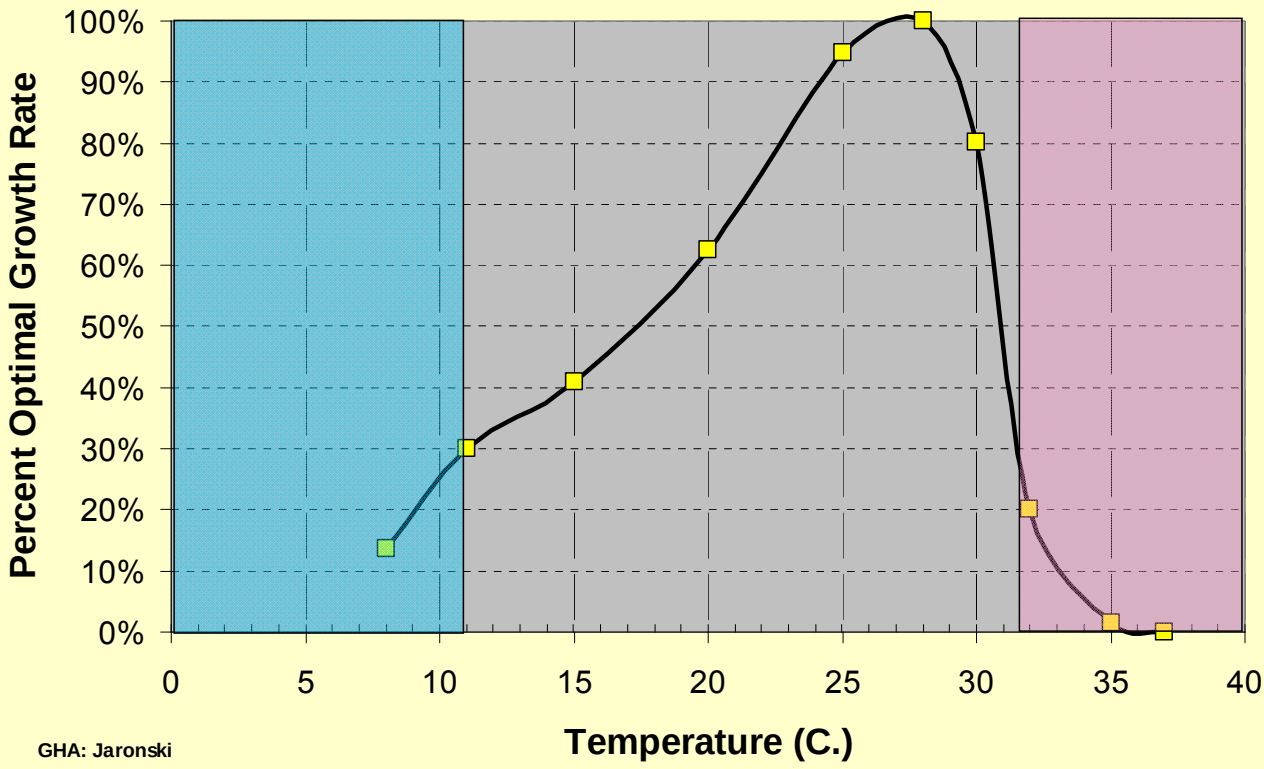
"Ecotolerances": Tolerance of an isolate to the important environmental variables in the target use arena.

- **Temperature**
- **Ultraviolet Radiation**
- **Humidity in target microclimate**

The ecological tolerances of microbial candidates, tolerances *relevant to the arena for which they are intended*, are also quite important.

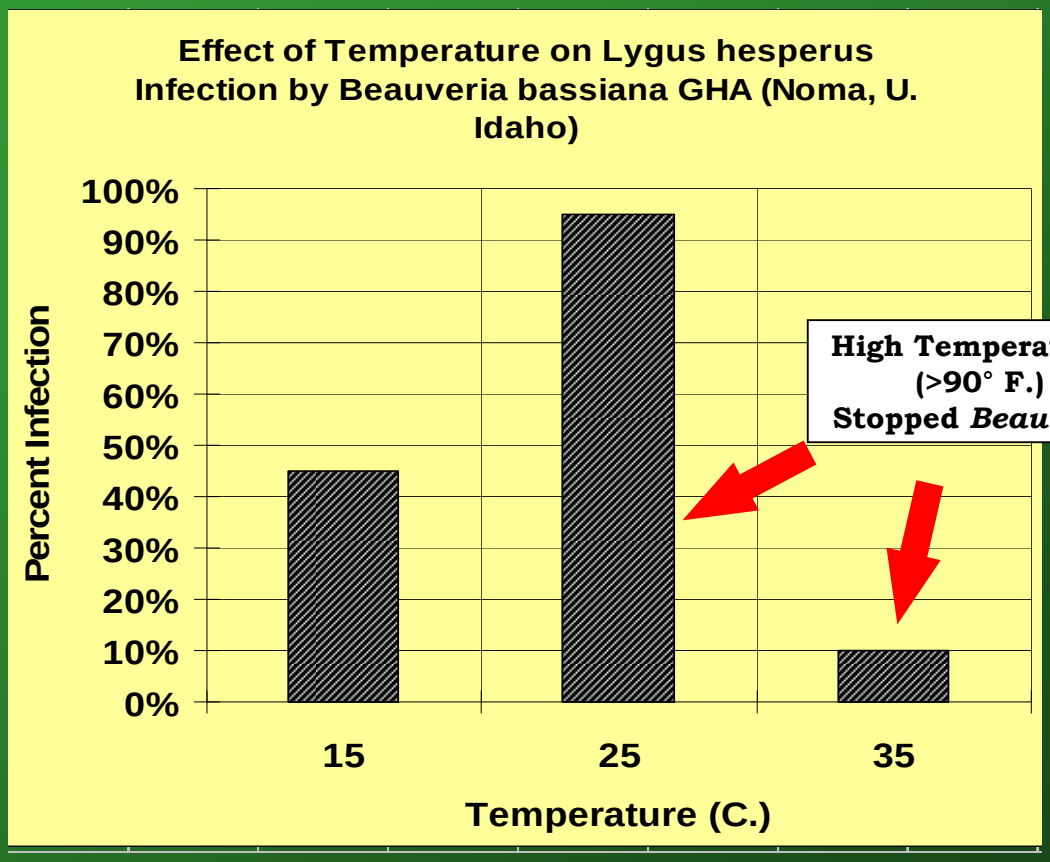
These include tolerance to

high or low temperatures
UV radiation
and perhaps also
Threshold water activity for spore germination



Here are thermal tolerances for Beauveria GHA, again. Note that it does not grow above 32-35 C.

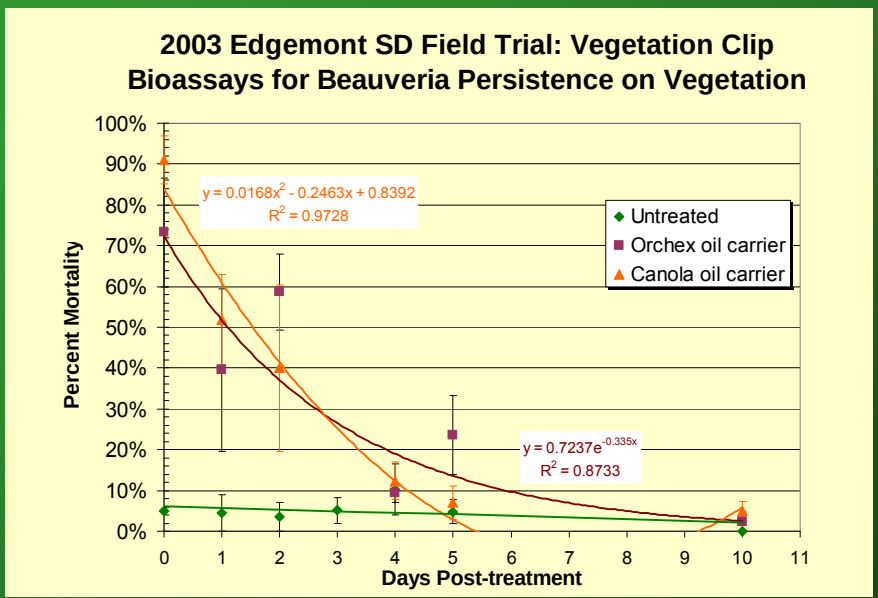
Effect of temperature on efficacy



Here's a real world example of the effects of temperature tolerances of a Beauveria (GHA) expressed by its infectivity and pathogenicity for Lygus.

When Takuji Noma failed to control Lygus in Alfalfa field trials he conducted some lab bioassays at 15, 25 and 35 C. At 35 C infection rates, and death from fungus, was much less than at 25 C, at the same dose of spores.

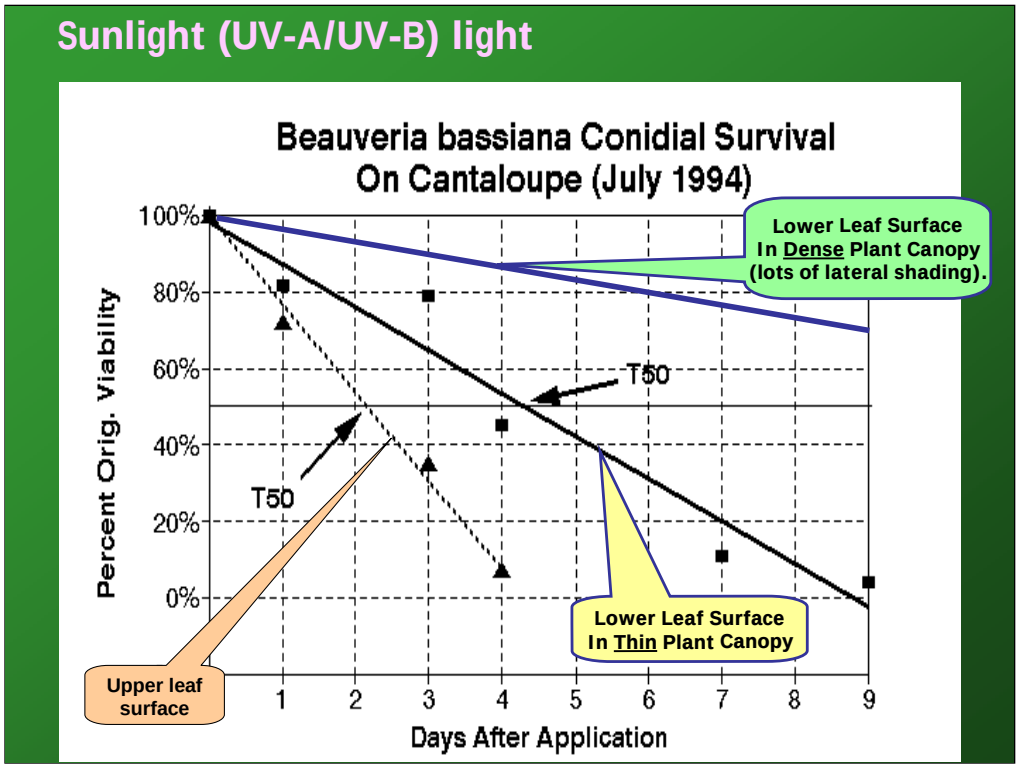
Sunlight (UV-A/UV-B) light



Jaronski & Foster unpublished

UV-A and UV-B are mortal enemies of fungus spores. Shown here is the degradation of Beauveria effectiveness during a grasshopper field trial on South Dakota rangeland. The residues of the fungus are rapidly killed off by UVso that half of the effectiveness is lost within 1-2 days of spray, and almost all effectiveness is lost after 6-7 days.

(Efficacy was measured by confining healthy grasshoppers for 48 hr with vegetation samples taken at specified intervals, then recording the mortality of those grasshoppers afdter 10 days.



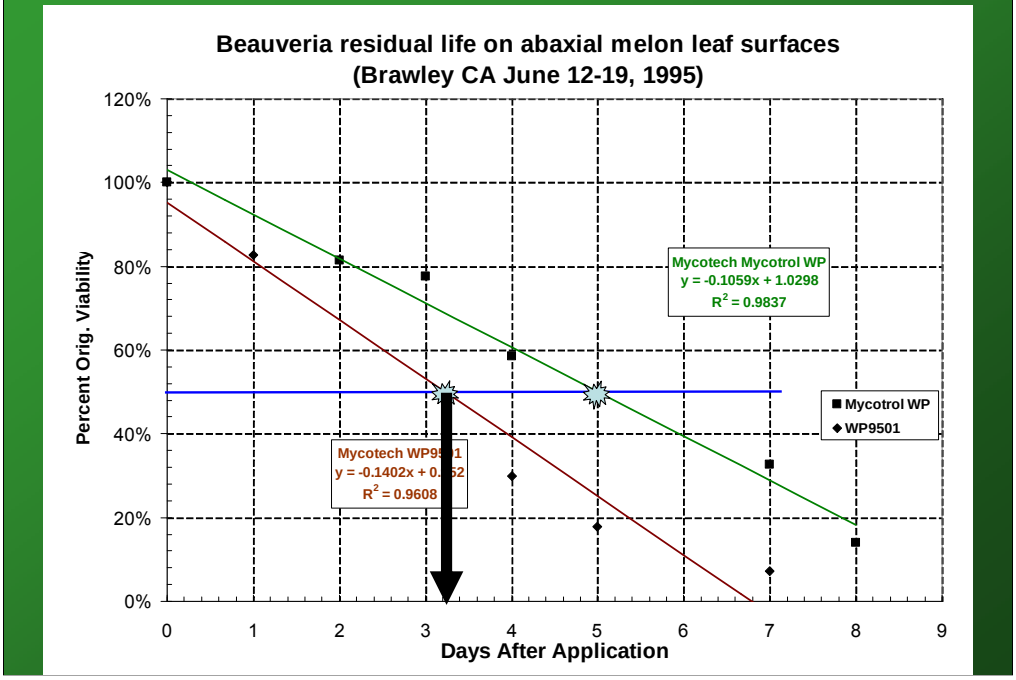
But life on the leaf surface can get complicated.

On the upper leaf surface half life of Beauveria is 2 days.

On the lower leaf surface, protected from direct sunlight and some blue-sky UV the spores had a half life for 4 days.

On the lower leaf surface, in a dense canopy, with lots of lateral shading by adjacent leaves, the spores were very pprotected with a half-life in excess of 14 days.

Formulation can provide some protection from UV



Formulation can provide some protection as seen here where the regular Mycotrol WP had a half life on melon leaves of a bit more than 3 days, while in an experimental WP formulation the half life was extended to 5 days.

A number of scientists have tried many UV protectants, but thos that have worked to any extent were either too expensive, impractical or toxic/carcinogenic.

UV protection still remains a Holy Grail

Effect of Humidity

High ambient relative humidity

- is NOT *always* required for infection
- IS required for sporulation.

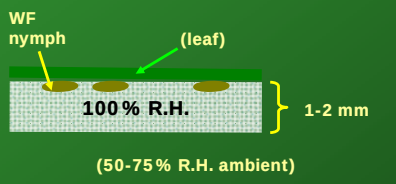
There is a conventional wisdom in my field that high humidity is required by these fungi for infection. That is true.

But one has to differentiate between ambient humidity (e.g., above a crop) and the microclimate humidity where the spore and insect interact, and infection takes place.

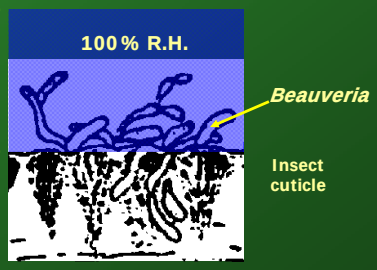
Ambient Humidity often has little effect

WHY?

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



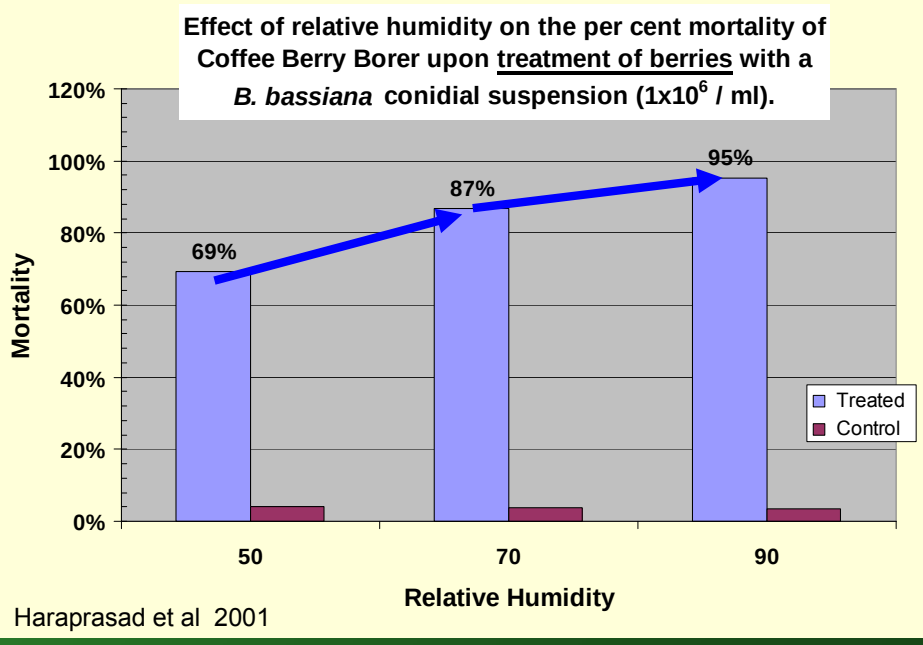
Cuticle of many larger insects has layer of high humidity



There is a boundary layer of still air 1-2 mm deep above leaf surface, esp. above the lower surfaces of leaves. Within that boundary layer humidity can be 100% even though ambient humidity is much lower. That's why Beauveria can be effective against whiteflies in a desert environment.

Wind has to exceed 10-15 mph before that boundary layer is stripped and humidity immediately adjacent to leaf become close to ambient.

What about CBB?

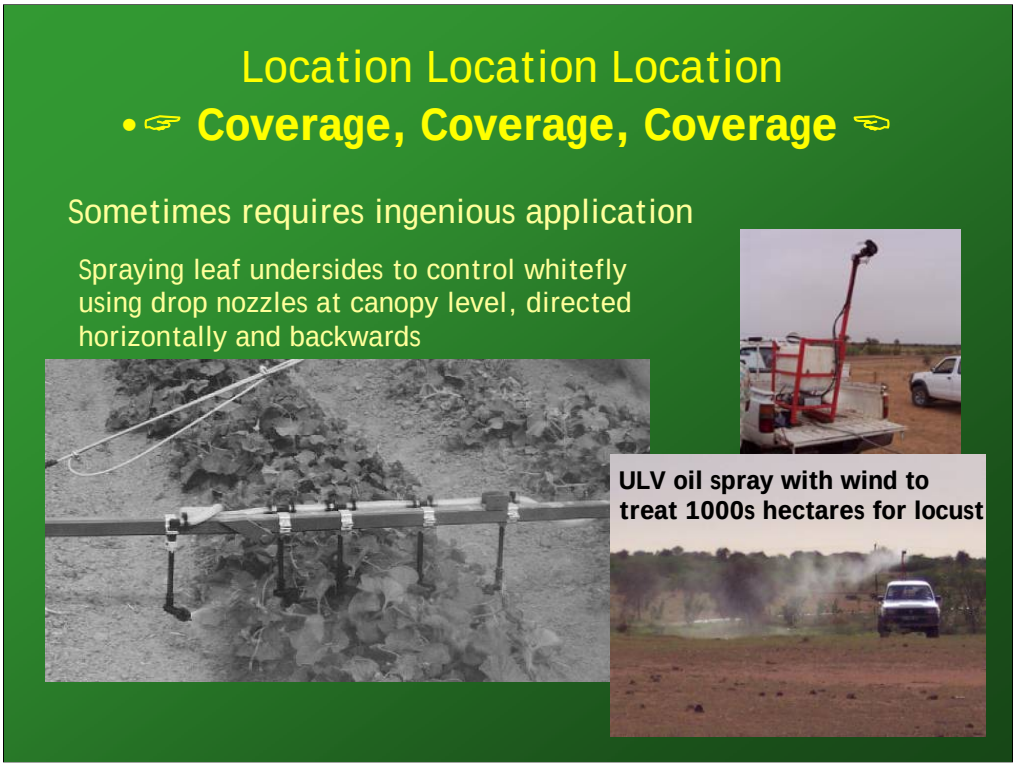


Haraprasad et al 2001

At least according to one study lower humidities do affect the efficacy of Beauveria for coffee berry borer. But at 50 and 70% RH, infection should not have occurred *at all* if the CBB microclimate humidity was close to those ambient levels! Thus the berries offer a higher humidity for infection of CBB

As a grower,
One has to think about these fungi in some new, different ways.

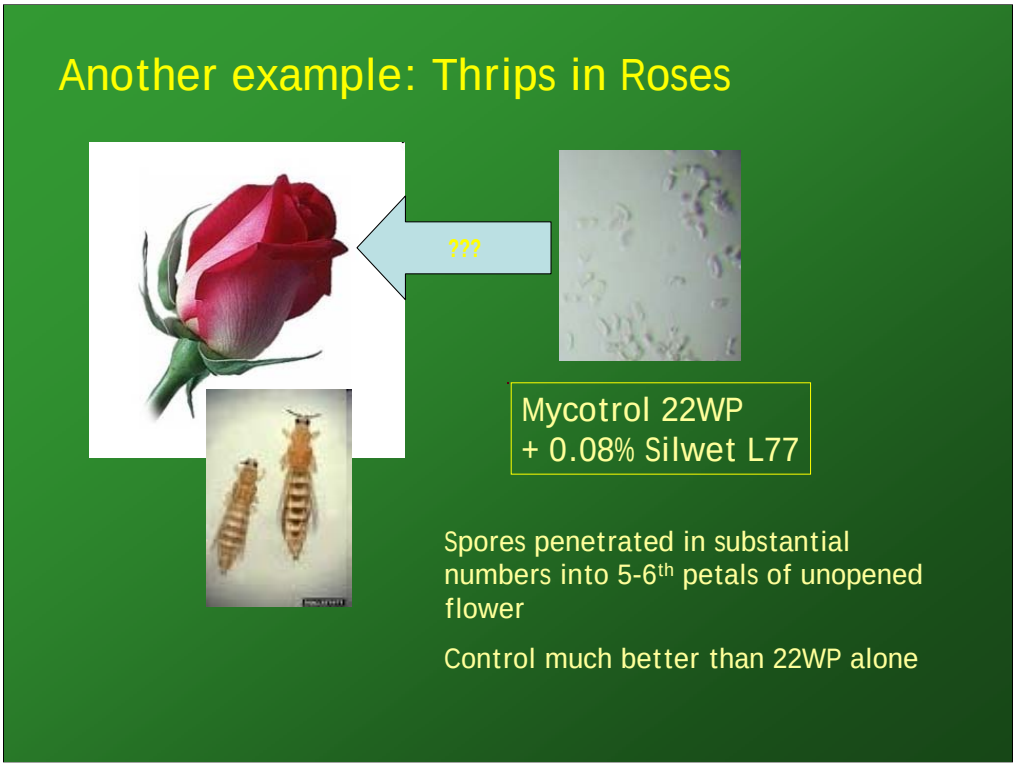
- Active Ingredient is a *living* organism - the fungal spore.
- *Direct contact* between spore and insect is necessary.
- Speed of Action is *slower* than chemical pesticides, more like an IGR.
  ☞ **One needs Patience** ☜
- These fungi **rarely recycle**; they are 'kamikaze' (coffee may be exception)
- **Concurrent pesticide use** a *potential* problem; care is needed



On the left is an ingenious way we developed to deliver Beauveria spores to the leaf undersides of melons, without any expensive add-on equipment.

The nozzles are on drop tubes 8 inches apart, just into the leaf canopy, and point horizontally and BACKWARD. In this manner the drop tubes act like tines of a comb, setting the umbrella-like leaves moving back and forth. The spray is directed horizontally with ~80% going to the leaf undersides. If the nozzles pointed forwards the leaves would block the spray, but backwards the leaves do not.

On the right is the manner is which Metarhizium is applied for locust control in Africa. That is a ultra low volume spinning disk sprayer on the back of a pickup truck. By spraying in a cross wind, one creates a fine fog of oil droplets with a swath of up to 100 feet, effectively contacting locusts with fungus spores. In this manner 1000s of acres can be treated by truck, using ½ to 1 quart of oil formulation per acre.



One more example: We were trying to deliver Beauveria spores to thrips in greenhouse roses in California. The adult thrips love to hide within partially opened rose petals.

We discovered that the superior spreading ability of Silwet L77 carried the spore suspension down into the 5th and 6th petals of an unopened rose! And gave good control.

Chemical Insecticide Compatibility Reference

Product	Manufacturer	Rate tested per 100 gal. of spray	Tank Mix Compatible?
Adept	Uniroyal	8 oz.	Yes
Avid	Novartis	8 oz.	Yes, but spray immediately
Azatin XL	Olympic Horticultural Products	21 oz.	Yes
Decathlon	Olympic Horticultural Products	1.9 oz.	Yes
Diazinon 4E	Novartis	16 oz.	Yes
Dursban 50W	DowElanco	32 oz.	Yes
Enstar II	Wellmark International	20 oz.	Yes
Evergreen Grower's Spray 7018	McLaughlin Gormley King Co.	210 oz.	***No***
Evergreen Grower's Spray 7405	McLaughlin Gormley King Co.	67 oz.	***No***
Garlic Barrier	Garlic Research Labs		Yes
Hexagon	Gowan Co.	2 oz.	Yes
Hot Pepper Wax	Wilder Agriculture Porducts	3% by volume	Yes, but spray immediately
M-Pede	Mycogen	2 gal.	Yes, mix BotaniGard First
Mavrick	Wellmark International	10 oz.	Yes
Metasystox-R	Gowan Co.	110 oz.	***No***
Neemazad	Thermo Trilogy		***No***
Neemazad 4.5	Thermo Trilogy	4.5 oz.	Yes
Orthene Turf, Tree, and Ornamental	Valent U.S.A. Corp.	21 oz.	Yes
Palmolive soap	Colgate-Palmolive	96 oz.	Yes
Pentac Aquaflow	Novartis	8.0 oz	Yes
PBO (piperonyl butoxide)	various	16 oz.	Yes
Pyrellin	Webb-Wright	32 oz.	Yes
Pyrenone	AgrEvo USA, Co.	12 oz.	Yes
Sevin 80S	Rhone-Poulenc, Inc.	48 oz.	Yes
SunSpray	Sun Refining and Marketing Co.	2 gal.	Yes
Talstar	FMC Corporation	40 oz.	Yes
Thiodan 3EC	FMC Corporation	22 oz.	Yes (up to 22 oz 3EC/100 gal)
Thiodan 3EC	FMC Corporation	64 oz.	***No***
Thiodan 50WP	FMC Corporation	24 oz.	Yes
Triact	Thermo Trilogy	2% by volume	Yes
Turcam	AgrEvo USA, Co.	42 oz.	Yes
Vendex	E.I. DuPont de Nemours and Co.	16 oz.	Yes
Vydate (Oxamyl) oil	E.I. DuPont de Nemours and Co.	32 oz.	Yes (up to 64 oz Vydate/100 gal)

Mycotech Corporation does not endorse the use of any specific listed insecticide. The grower should make their own choice based on their individual circumstances.

In using a fungus in conjunction with chemical pesticides, one has to be careful not just with the chemical but also different formulations of the same chemical.

Here, Neemazad was not compatible with Beauveria GHA but Nemmazad 4.5 was.

Similarly Thiodan 3EC at a low concentration was OK as was the WP, but not at the high label rate.

There is little value in guessing compatibility. One has to determine it experimentally.

Fungicide Compatibility Reference

Fungicide	Brand Tested	Manufacturer	BotaniGard Compatibility
TANK MIX			
Ampelomyces quisqualis	AQ10®	Ecogen Inc.	Tank-mix
Copper Lineolate	Tenn-Cop 5E®	Boliden Intertrade Inc.	Tank-mix
Copper Hydroxide	Kocide DF®	Zeneca	Tank-mix
Copper Sulfate	Phyton 27®	Source Technol. Biol.	Tank-mix
Thiophanate-methyl	Cleary's 3336 WP®	WA Cleary Chemical Corp.	Tank-mix
Fosetyl-Al	Aliette WDG®	Rhône Poulenc Ag. Co.	Tank-mix
SAME DAY			
Sulfur - volatilized	various	various	Same Day
TWO DAYS			
Sulfur - liquid	various	various	2 days before or after
Iprodione	Chipco 26019®	Rhône Poulenc Ag. Co.	2 days before or after
Myclobutanil	Rally 40W®	Rohm and Haas Co.	2 days before or after
Propiconazole	Banner Maxx	Novartis	2 days before or after
Piperalyn	Pipron®	SePro Corp.	2 days before or after
Triadimefon	Bayleton®	Bayer	2 days before or after
Vinclozolin	Ornalin®	BASF Corp.	2 days before or after
Thiram	Spotrete®	WA Cleary Chemical Corp.	2 days before or after
Triforine	Funginex®	Novartis AG	2 days before or after
THREE DAYS			
Chlorothalonil	Daconil®	Zeneca	3 days before or after
Metalaxyl & Chlorothalonil	Subdue® & Daconil®	Novartis AG & Zeneca	3 days before or after
FOUR DAYS			
Azoxystrobin	Quadris® & Hertage®	Zeneca	4 days before or after
Benomyl	Benlate®	Dupont De Nemours and Co. (Inc.)	4 days before or after
Captan	various	various	4 days before or after
Fludioxonil	Medallion	Novartis	4 days before or after
Maneb	Maneb 80®	Elf Atochem NA	4 days before or after
Thiophanate/Mancozeb	Zyban®	Scotts Co.	4 days before or after
Triflumizole	TerraGuard 50W	Uniroyal	4 days before or after

You would think that many/most fungicides would be death for aBeauveria. But not so.

Unlike the usual academic approach in which the fungicide is incorporated into an agar medium and the the fungus added, we looked at the effect of residues on the leaf surface. We applied each of these fungicides 0, 2, 3, or 4 days before we applied Beauveria. We then incubated the spores on the leaves a few hours and washed them off and determined spore viability.

As you can see here some otherwise harmful fungicides (in a lab assay) can be applied 2-4 days before the Beauveria without harming efficacy of the fungus. In some cases the fungicide is rapidly absorbed into the leaf cuticle so that there really are no residues when the Beauveria spores land on the leaf surface.

Other factors affecting Beauveria efficacy

- Physical & chemical nature of leaf surface
- Leaf expansion diluting conidia concentration, or creating new, untreated surfaces
- Insect behavior (species differences, stadium differences)
- Tritrophic interactions (plant allelochemics acquired by insect in feeding alter susceptibility (+ or -) to fungus infection)

There are other factors that can affect the efficacy of Beauveria ...

Some studies have revealed that chemical on the leaf surface can inhibit spore germination, or presence of fine leaf hairs prevent physical contact between spores and insect. Beauveria does not work very well against thrips on impatiens but does on beans

Rapidly expanding leaf canopies create areas of leaves not treated with fungus necessitating repeat spraying even as often as every 5-7 days. This is case with melons and cantaloupe where whiteflies like a certain age younger leaf to lay eggs so that one is spraying fungus onto a “moving target.”

As I mentioned before species differences, stadium differences in behavior can affect effectiveness of a fungus.

Lastly insects can pick up substances that can make them more resistant to infection (alkaloids in potato and green peppers), or that can stress the insects and make them more susceptible to infection (leaf tannins)

What about Beauveria for CBB?

- found in all the coffee regions infested by CBB
- main natural mortality factor of CBB
  - Venezuela: 30% mortality (Klein Koch et al., 1988),
  - India: 60% in India (Balakrishnan et al., 1994),
  - Mexico: <10% (Méndez-López, 1990 ; Cordova-Gámez, 1995),

Beauvreia has been seen in CBB populations all over the world

It is a natural enemy of CBB and can cause high mortality at times

Points of attack

- Green berries on the tree
  - When adults are migrating to and boring into berries
  - Adults and immatures inside berry

  Needs superior carrier e.g., Silwet Eco Spreader
- Infested berries on the ground

  Needs superior carrier e.g., Silwet Eco Spreader

So how can one use Beauveria to manage CBB populaitons???

OK Now the perspective of an unmarried marriage counselor – I'm not the CBB expert -- on how one can attack the insect.

In both cases a superior wetting agent such as the OMRI-certified Silwet Eco may bee the key to success. Silwet so lowers surface tension of water that spores should be carried into the tunnels and galleries in the berry, as well as all the nooks and crannies in the berry cluster, and into good contact with the insect.

An organosilicone spreader is the key to wetting the coffee trees properly with spray using a minimum volume. I have used it in a number of crops to successfully deliver spores to the target insect.

Effect of *Beauveria* conidial suspension ($1x10^6$ conidia/ml) on the mortality of CBB and berry infestation (India)

	CBB mortality (%)	Infestation of coffee berries (%)	
	Sprayed *	Sprayed	Unsprayed
Sep 1995	**80.5 +/-0.07**	**1.5 +/-0.05**	9.7 +/-0.10
Jan 1996	**64.5 +/-0.10**	**1.8 +/-0.07**	20.5 +/-0.20
Sep 1996	**80.4 +/-0.08**	**2.4 +/-0.05**	9.7 +/-0.10
Jan 1997	**65.4 +/-0.12**	**1.6 +/-0.09**	19.3 +/-0.14
Sep 1997	**79.3 +/-0.17**	**2.5 +/-0.10**	9.8 +/-0.04
Jan 1998	**77.5 +/-0.15**	**1.8 +/-0.06**	18.3 +/-0.09
Sep 1998	**80.3 +/-0.06**	**2.2 +/-0.10**	9.7 +/-0.08

* Percent mortality in unsprayed plots was zero in all the seasons.

Values are the means of five independent experiments +/- SE in each season.

infested coffee berries sprayed to run-off, 2L per bush

Haraprasad et al. 2001

The mycopesticide (1 $x10^6$ conidia /ml) was prepared by suspending 1 g of the lyophilized fungal culture in 5 l of water and spraying infested coffee berries to run-off using locally-available 'Gator' rocking sprayer. For 200 plants of coffee, 20 g of the lyophilized conidial mass was suspended in 100 l of water and used for spraying.

CBB mortality was substantial in all years and Percent infestation of berries was reduced significantly (although the highest untreated infestation level was 20%).

Additional cause for hope

- 1 spray of 10^9 *Beauveria* spores/tree
  → ~20-40% prevalence for 60 d
  (De la Rosa et al 2000)
- 3 sprays *Beauveria* (@ 0, 25, and 42 d.)
  →40-50% rate for 60 d
  (Baker 1999)
- 6 applications of *Beauveria* → 60-70% infection for 119 d
  (Baker 1999)

Here's some more data for different *Beauveria* strains, drawn from Latin American studies

(In comparison a quart of Mycotrol O contains $2x10^{13}$ spores.)

How does one spray coffee?

So how does one apply Beauveria to coffee to attack the CBB?

On farms like this, where the trees are well spaced, and there are alleys between rows of trees, there is plenty of access to the trees.

5 m vertical, 10 m horizontal;
600 L tank

Container Capacity: 400,600,-800L
Level range: ≥ 40 m
Vertical range: ≥ 30 m
Pump Type: diaphragm
Mating Power: 70 horsepower tractors
Work efficiency: 600 acres / hour (10 km per hour)
300 acres / hour (5 km per hour)

Therefore a small orchard blast sprayer will work just fine

But what about farms like these?

But on farms where the trees are in dense stands and it is difficult to move around them, it's a different story. Orchard sprayers won't work (unless you can levitate them above the ground!).

In such cases, based on my experience in many different crops, a backpack, motorized mist blower may be the best sprayer.

Regular backpack sprayers have uneven pressure which causes uneven spray, don't penetrate canopy readily or evenly, and can too often casue tendency to spray too much onto the trees, wasting fungus.

Figure 3 - Average Particle Size Distribution

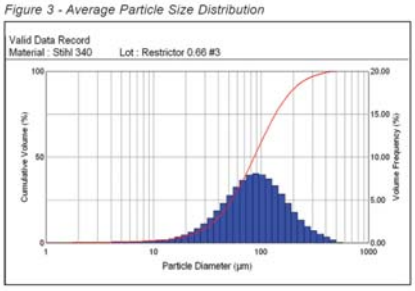


Table 1

Sprayer	Engine Speed (rpm)	Air Speed (m/s)	Air Volume Flow Rate (m3/h)	Vertical Throw (m)	Horizontal Throw (m)
Solo 423	5600	97.7	690	8	12
Solo 450	5500	92.6	654	10	15
Stihl 340	6550	90.6	892	7	13
Stihl 420	6950	98.0	965	11	15
Guarany 54M ULV	7000	96.4	1534	10	15

The motorized mist blower puts out a fine, penetrating mist at high velocity, and extend reach of the spray 7-15 meters. This allows the applicator to stand outside a “coffee tree jungle” and spray the trees thoroughly.

Such sprayers are routinely used in cacao and coffee in Africa and South America.

And then you can treat your homes for roaches and bedbugs...

(Photo of US Army treating barracks to kill bed bugs with a pyrethroid.)

Using motorized mist blower

- Calculate flow rate ...
- Flow rate: 0.33 liters (11.5 oz) per minute
- If time required to treat individual tree is 0.17 minute (10 seconds)

  ➔ 0.06 liters (0.064 qt, 1 fl oz) spray per tree; = 166 trees per 10 L tank

- If 500 trees per acre, then = 30 liters per acre (8 gal/acre); 3 loads of backpack mist blower

(10 seconds may seem a very short time, but it is probably sufficient time to properly spray a coffee tree with a motorized mist blower. (Try it ... Count steadily and slowly, “One thousand, two thousand, three thousand ... Ten thousand.”)

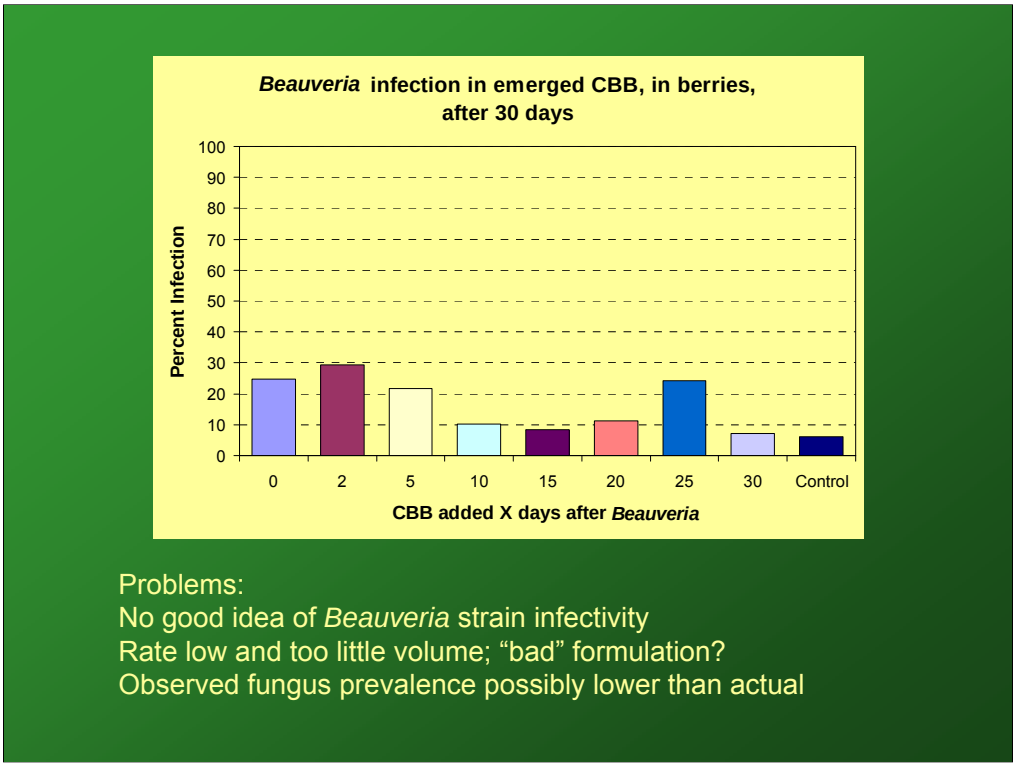
Will CBB emerging from treated infested berries contact the fungus and become infected?

- With rainy season massive adult emergence occurs
- High humidity caused by rainfall is the main trigger of CBB emergence from fallen berries.
- Soil moisture stimulates expulsion and death of the immature stages inside the berry (Baker et al. 1994).

☞ Target the fallen berries to infect CBB before and during emigration from berries

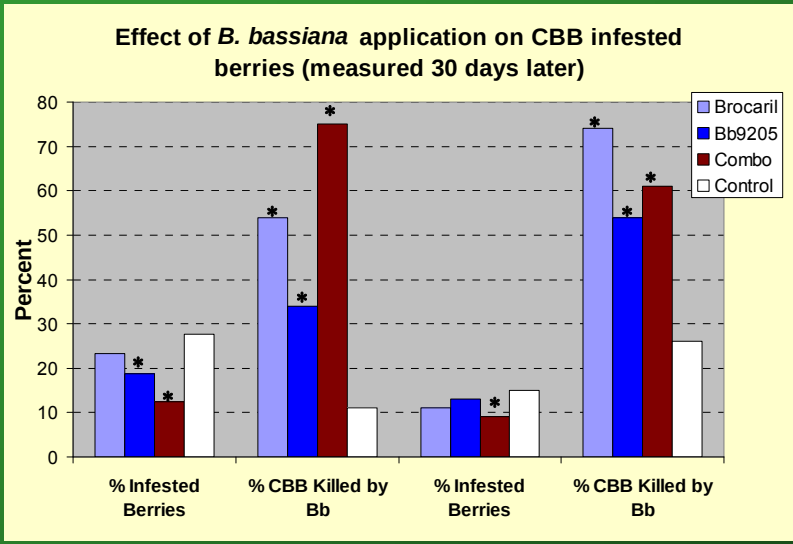
Bustillo et al. (Florida Entomologist 82(4). 1999)

- Added CBB infested berries to ground beneath trees
- *Beauveria* sprayed on ground at base of trees
- Spores in Tween-20 and "emulsified oil carrier" (1:1) then diluted in water
- $2x10^7$ conidia/ml in a volume of 50 ml/tree with a manual backpack sprayer
- final dose of $1x10^9$ conidia/tree.



Results were not very good, but there were problems with the study

More recent research (in Colombia):



50 mL of a $2x10^{10}$ conidia/L conidia suspension sprayed to each tree base, for a dose of $1x10^{9}$ conidia/base; plots caged

Vera et al Biocontrol Science and Technology, Vol. 21, No. 1, 2011, 114

A better study at two different sites (left and right) in South America, was just published,

The rate, $2x10^{10}$ conidia/L = 0.12 fl oz Mycotrol O / gal spray

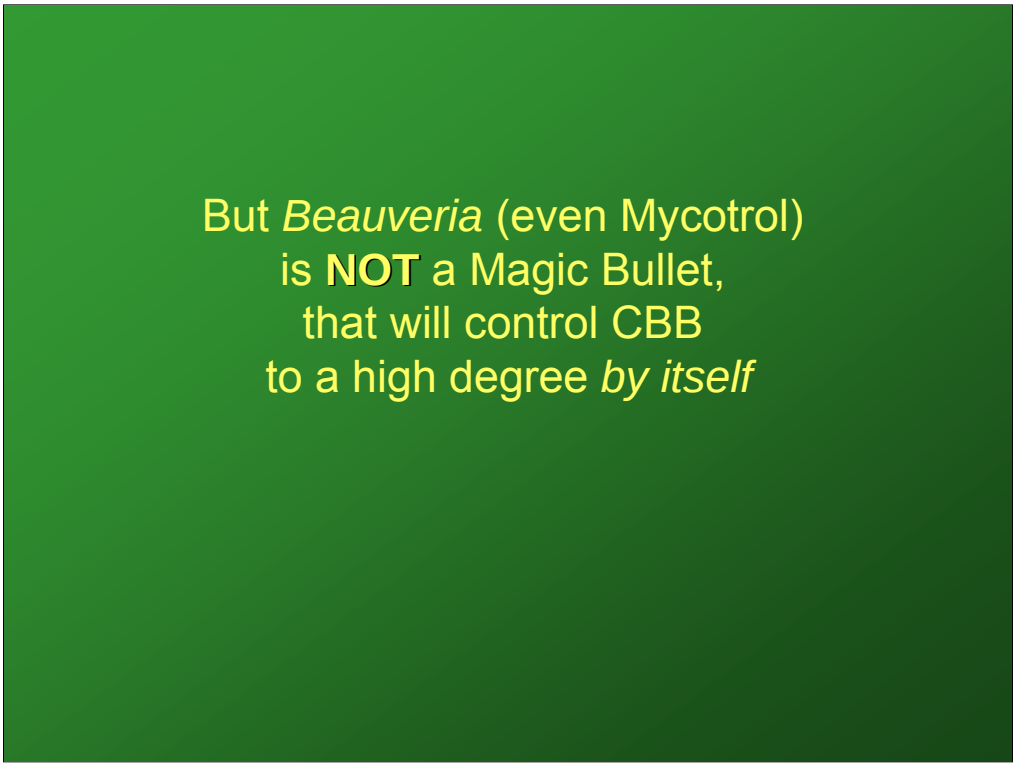
(but we do NOT know how effective the Beauveria GHA in Mycotrol is for CBB. Hawaii Dept of AG and USDA have to determine the best rates.)

The star above each bar indicates that that percentage of CBB killed or % infested berries was significantly different from the untreated control.

So ground sprays were efficacious in killing beetles and did lower bean infestation somewhat (but could you live with the lower infestation here?)

But is treating the ground really feasible in Kona?

But how effective would a ground spray be on Hawai'I, with ground like this beneath the trees? Where berries fall in between the lava chunks and are hard to reach with a spray? That remains the question.



My most important message! Please read it aloud three or more times

PLEASE note,

We should NOT use
microbials
like chemical insecticides

simply substituting for a
chemical

More fundamentally, ...

In transient, annual crops,
Microbials can't do the job alone,

- Rarely achieve >80% efficacy
- These fungi rarely recycle
- Finite residual life on plants
- "Epidemic math" (spores/cm^2 leaf), must be overcome
  by enough spores and application frequency
- Slow action on hungry insects, rapidly multiplying insects

In perennial crops (coffee),

Microbials can do better,

The fungi can recycle if it's humid enough (coffee?)

BUT,

- Finite spores have residual life on plants
- "Epidemic math" must be overcome
  by enough spores and application frequency
- Slow action on hungry insects,
  rapidly multiplying insects
- Rarely achieve >80% efficacy

Microbials need to be applied at or even before the economic threshold --

the pest level at which control should be made to *prevent an increasing pest population* from reaching the economic injury level.

NOT to suppress an existing outbreak

We have to remember

The goal should be

Fire Prevention
not
Fire Extinguishing

… is very important!

Integrated Pest Management:

Use *multiple* tools *to manage* a pest population at a *lower average level* than would otherwise occur.

So what's the answer if Beauveria isn't good enough by itself?
INTEGRATED PEST MANAGEMENT

And IPM for CBB?

"We suggest that the use of *B. bassiana* should be incorporated in an

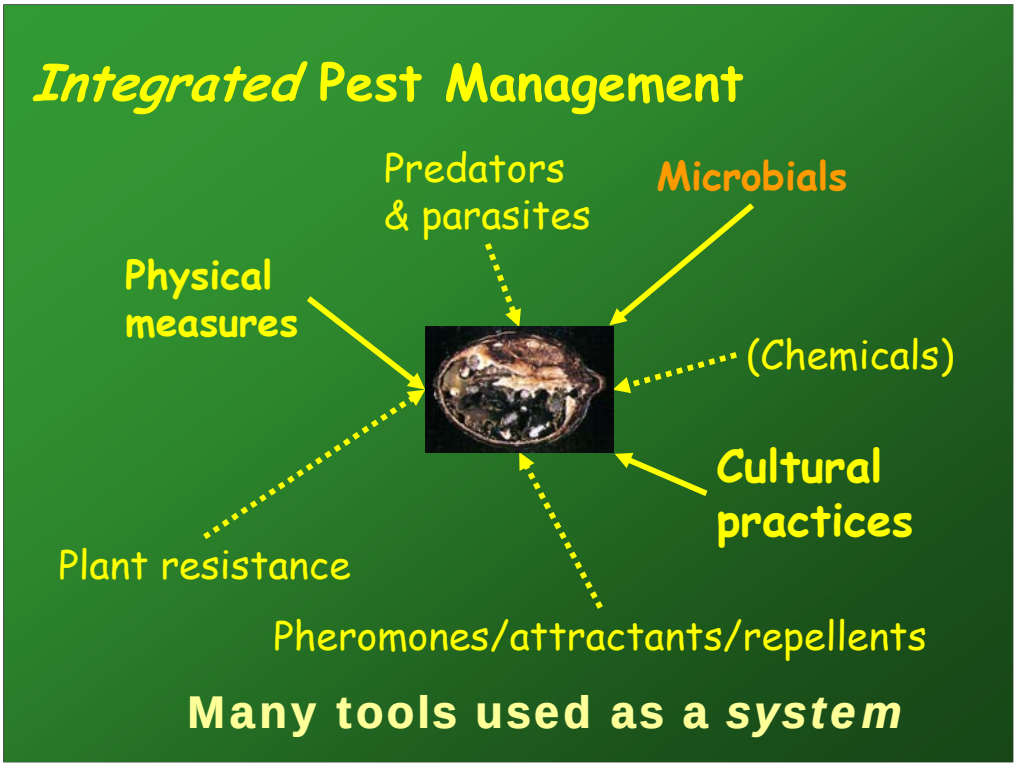
integrated pest management strategy

in such a way that the
moderate levels of mortality caused by the fungus are complimented by cultural control (collection and elimination of berries infested by the pest)

and other biological control agents such as the parasitoids."

De La Rosa et al 2000

Here's what the Columbian CBB experts write,



So the key is to use a number of different tools, each of which by itself may not be good enough, in combination so that the overall effect is efficacious in managing the CBB and keeping it at levels that will allow you to grow a profitable crop.

For CBB those tools, right now, are

•Physical Measures

•Cultural Practices

•Beauveria

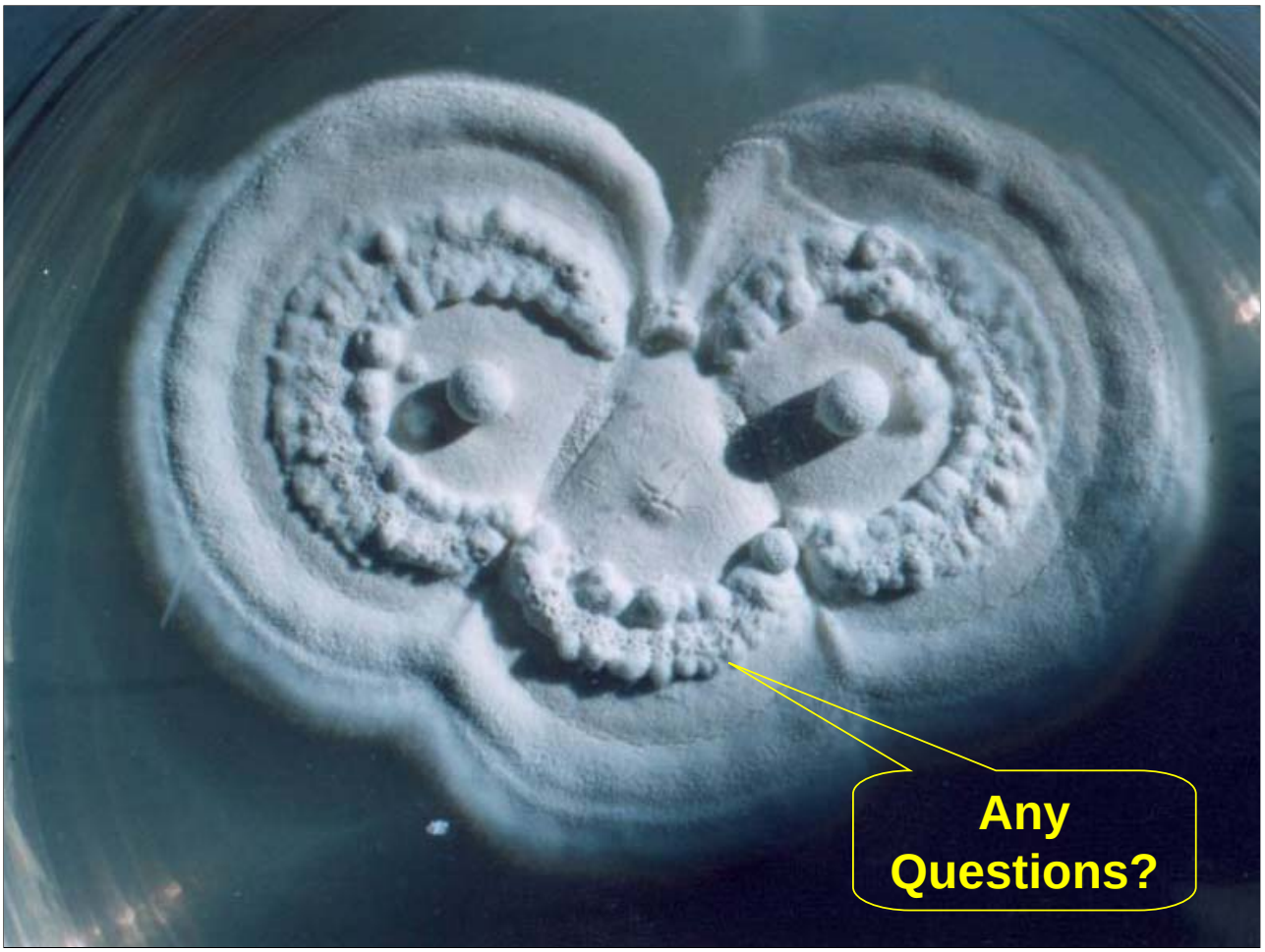
In the future

maybe

•predators and parasites

•Attractant traps or pheromone signal disruptors

•Resistant/tolerant coffee varieties


Any
Questions?