College Of Computer, Mathematical and Natural Sciences

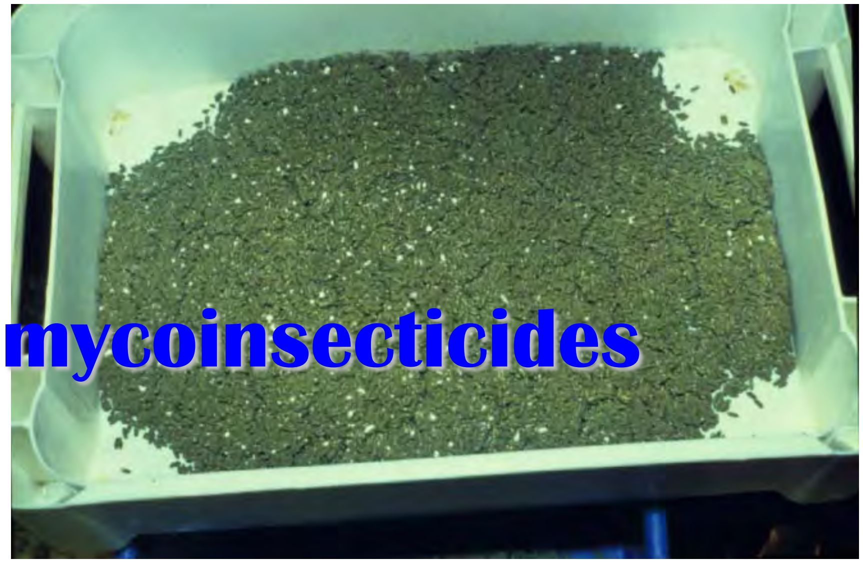
Department of Entomology

University of Maryland

Fungal Pathogens *Metarhizium*

Raymond J. St. Leger

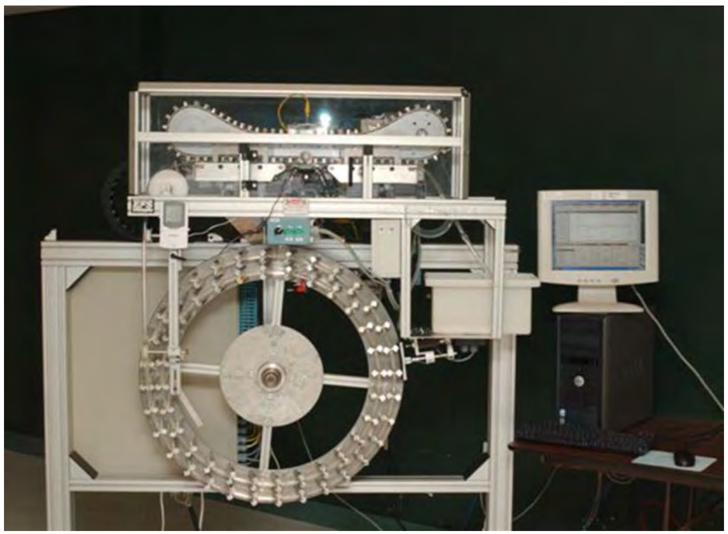
In terms of using fungi for control of BMSB we don't need to reinvent the wheel. Industry has the technology for mass production and distribution. *Metarhizium* is also produced by farmer's collectives in many parts of the World using low cost solid media such as barley, wheat, rice, broken maize and syrup.



Mass production of mycoinsecticides

Since 2007, *Metarhizium acridum* has been used ～ 200,000 hectares annually for locust control.

- Three basic strategies for selection and/or improvement of biological control agents:
  (1) find natural isolates
  (2) directed adaptation (Mutagenesis and/or Evolugator)



(3) genetically engineer improvements

Appl Microbiol Biotechnol (2014) 98:777–783
DOI 10.1007/s00253-013-5360-5

APPLIED GENETICS AND MOLECULAR BIOTECHNOLOGY

Overexpression of a *Metarhizium robertsii* HSP25 gene increases thermotolerance and survival in soil

Xinggang Liao · Hsiao-Ling Lu · Weiguo Fang · Raymond J. St. Leger

environmental microbiology
sfam
Environmental Microbiology (2010) 12(3), 810–820 doi:10.1111/j.1462-2920.2009.02127.x

RNA binding proteins mediate the ability of a fungus to adapt to the cold

Weiguo Fang* and Raymond J. St. Leger
University of Maryland, Department of Entomology, 4112 Plant Sciences Building, College Park, MD 20742, USA.

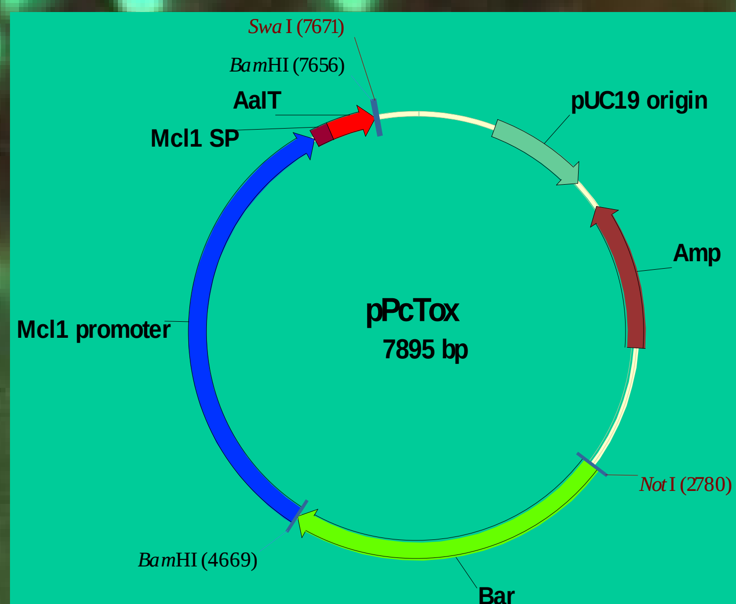
biology, they cannot elucidate the mechanisms involved in major fungal lifestyles they do not share. This matters because the fungal kingdom, with an estimated 1.5 million different species, displays extraordinary evolutionary

New spider toxins being expressed in *Metarhizium*

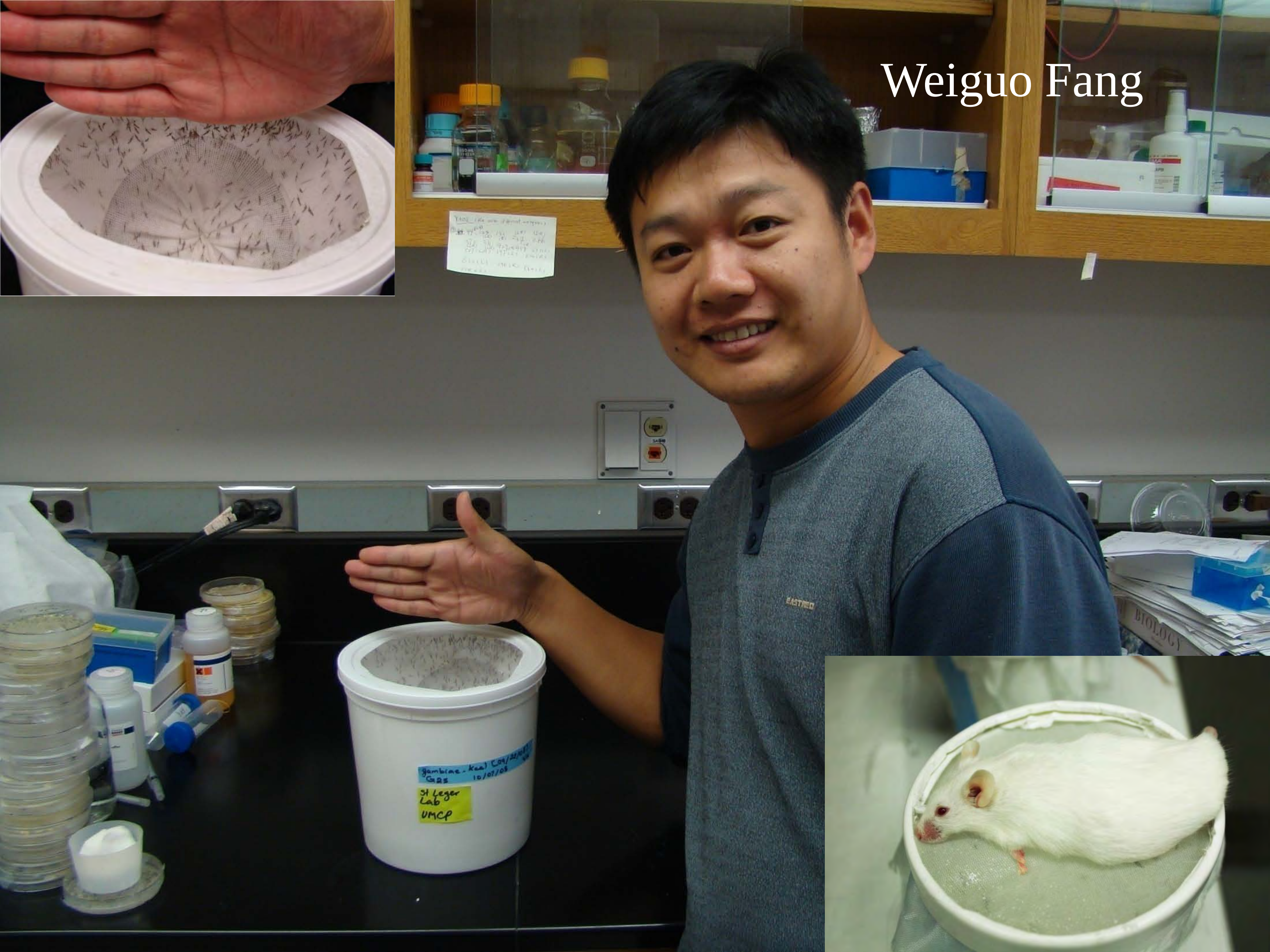
Many novel spider toxins have been transformed into *Metarhizium* strains targeting malaria vector *Anopheles gambiae*, different orthopterans and, we'd hoped, bugs.

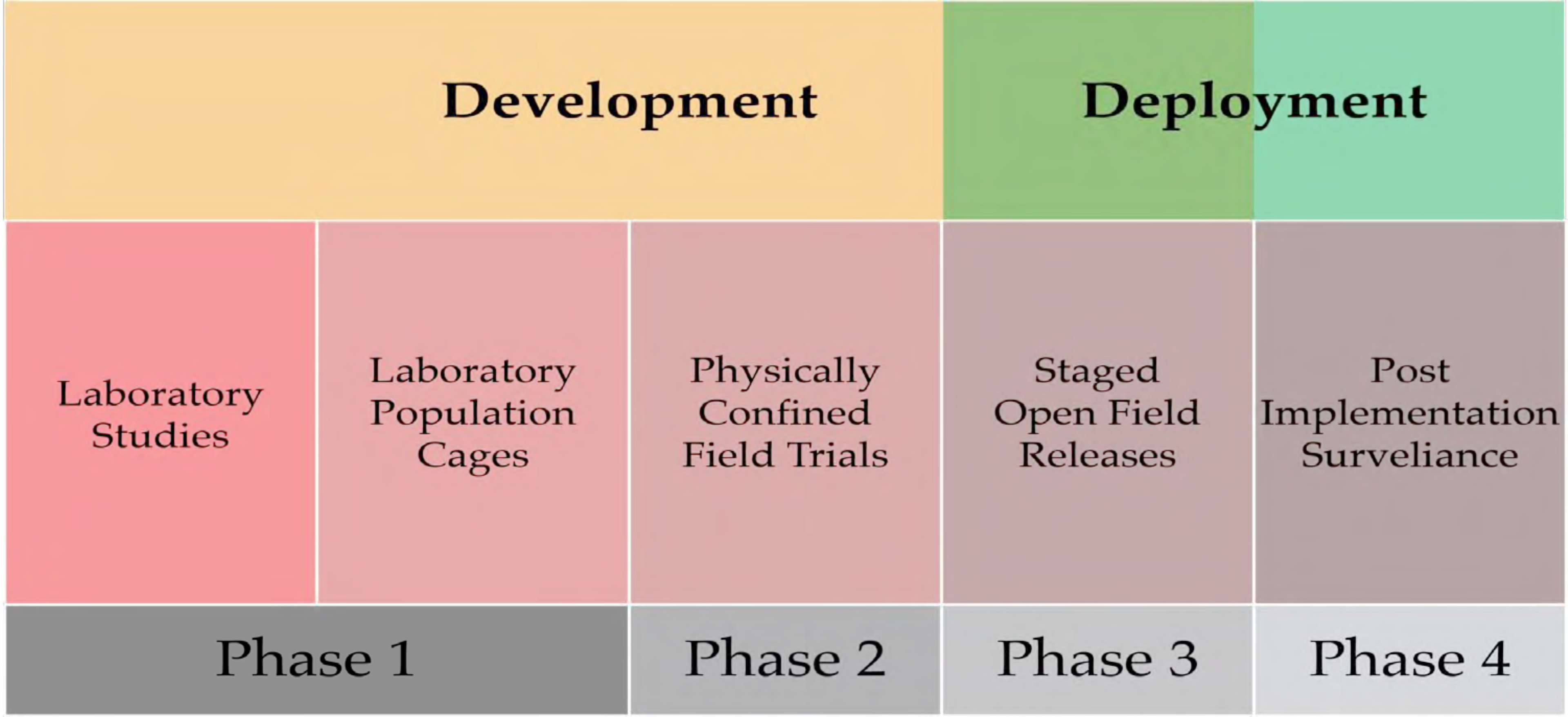
List of toxins that are very effective against one or more pest Insects:

As1a
Dc1a
Hv1d
Ta1a
Hybrid



Niraj Bende, Hsiao-Ling Lu


Weiguo Fang
St Leger
Lab
UMCP



The transition from one phase to the next is subject to "go/no-go" decision criteria

- Efficacy and safety endpoints
- Obtain regulatory approvals
- Social acceptance.







The construction of the Malaria Sphere
was funded by the United States National Institute
of Allergy and Infectious Diseases,
part of the National Institutes of Health-August 2014

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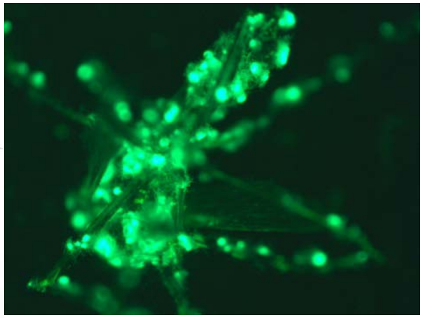
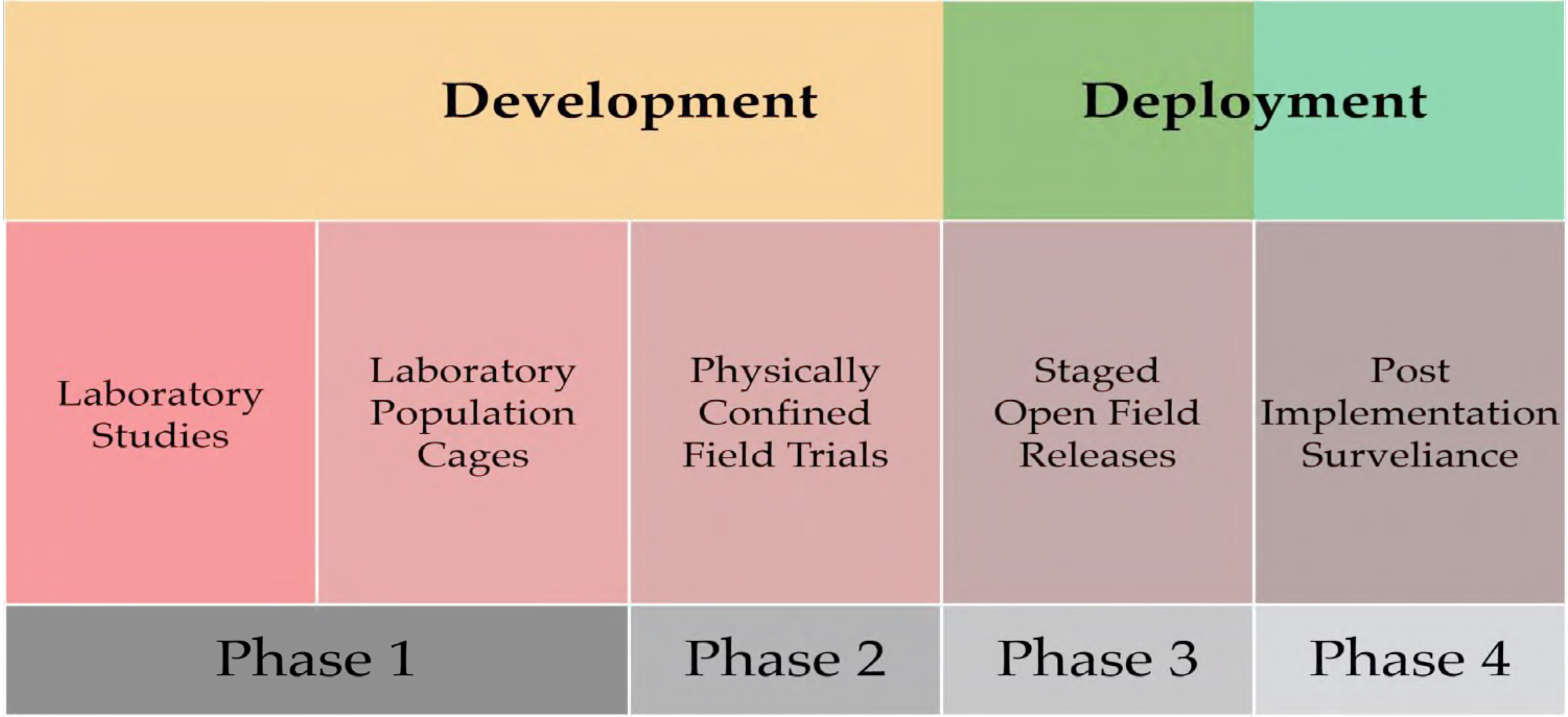




The transition from one phase to the next is subject to "go/no-go" decision criteria

- Efficacy and safety endpoints
- Regulatory and ethical approvals
- Social acceptance.



Oil-palm plantations now cover millions of hectares across Africa, Asia and South America and are a major source of income for smallholder growers. >80% of pest problems in PNG derive from Sexava tettigonids (long horn grasshoppers). Methamidophos is ineffective and dangerous. PNG-OPRA is committed to RSPO principles, recognizing the urgent and pressing need for a more eco-conscious approach to oil palm production. We have produce hypervirulent fungi that selectively kill these grasshoppers.

Production of a viable model for the development of GM *Metarhizium* as locust control tools.

The potential for direct financial returns uncertain (especially with self sustaining versions). Small companies, public-private partnerships, non-profit corps, and most importantly technology transfer to afflicted countries.

OPEN

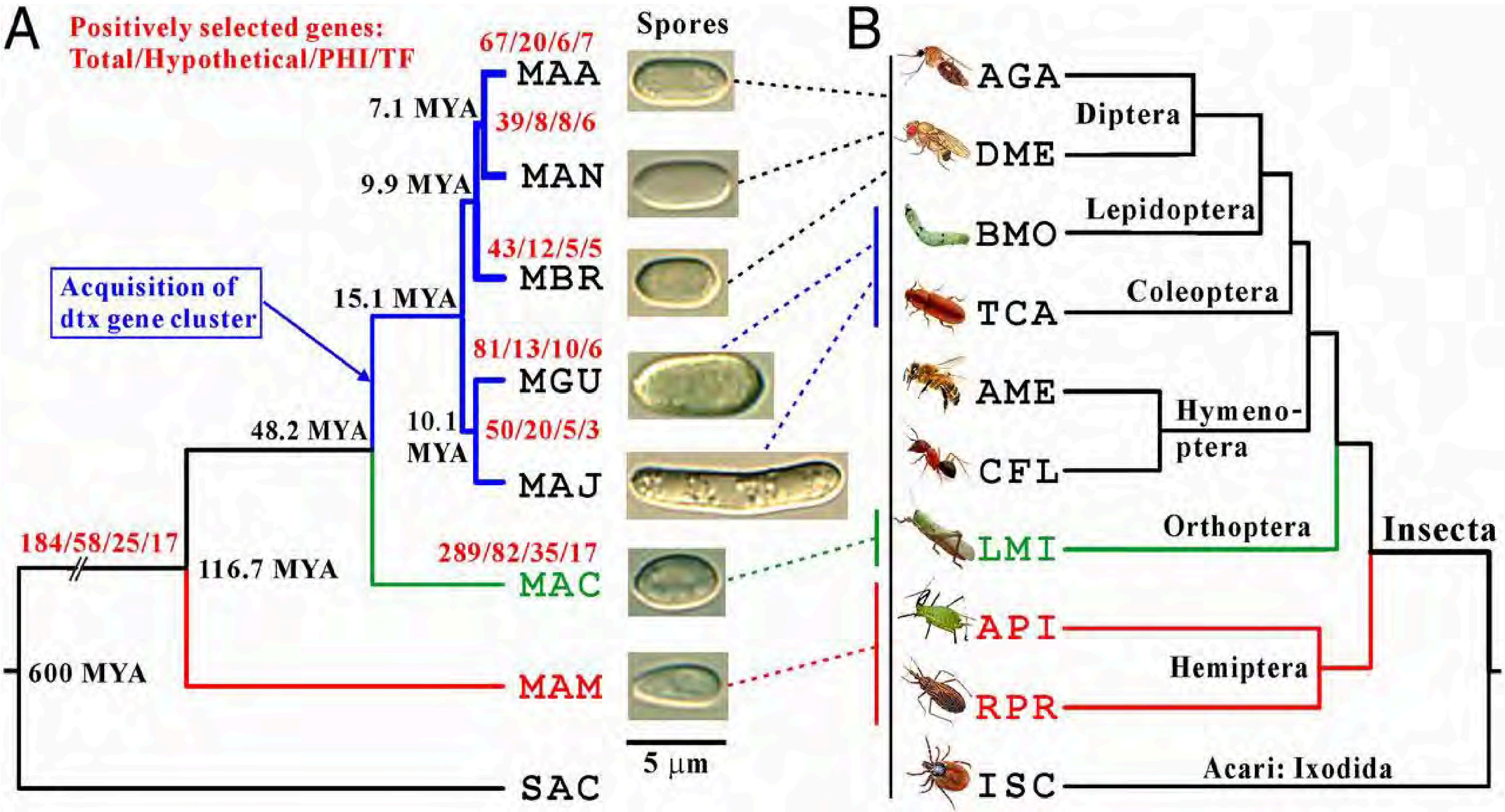
SUBJECT AREAS:
APPLIED MICROBIOLOGY
MOLECULAR ENGINEERING

Construction of a Hypervirulent and Specific Mycoinsecticide for Locust Control

Weiguo Fang[1], Hsiao-Ling Lu[2], Glenn F. King[3] & Raymond J. St. Leger[2]


Previous work

- **Several strains of entomopathogenic fungi assayed against adult and nymphal brown marmorated stink bug**
- **Poor performance of fungi**
  - **Low mortality**
  - **Low fungal growth/sporulation**
  - **Low virulence**



Hu et al. "Trajectory and genomic determinants of fungal-pathogen speciation and host adaptation." *Proceedings of the National Academy of Sciences* 111.47 (2014): 16796-16801.

Pre-penetration barriers

The pirate bug *Anthocris nemorum* avoids foraging and ovipositing on leaves containing spores of *Beauveria bassiana* (Meyling and Pell, 2006, Ecol. Entomol. 31, 162-)

Gland secretions and the microbial community add antibiotic properties to the cuticle

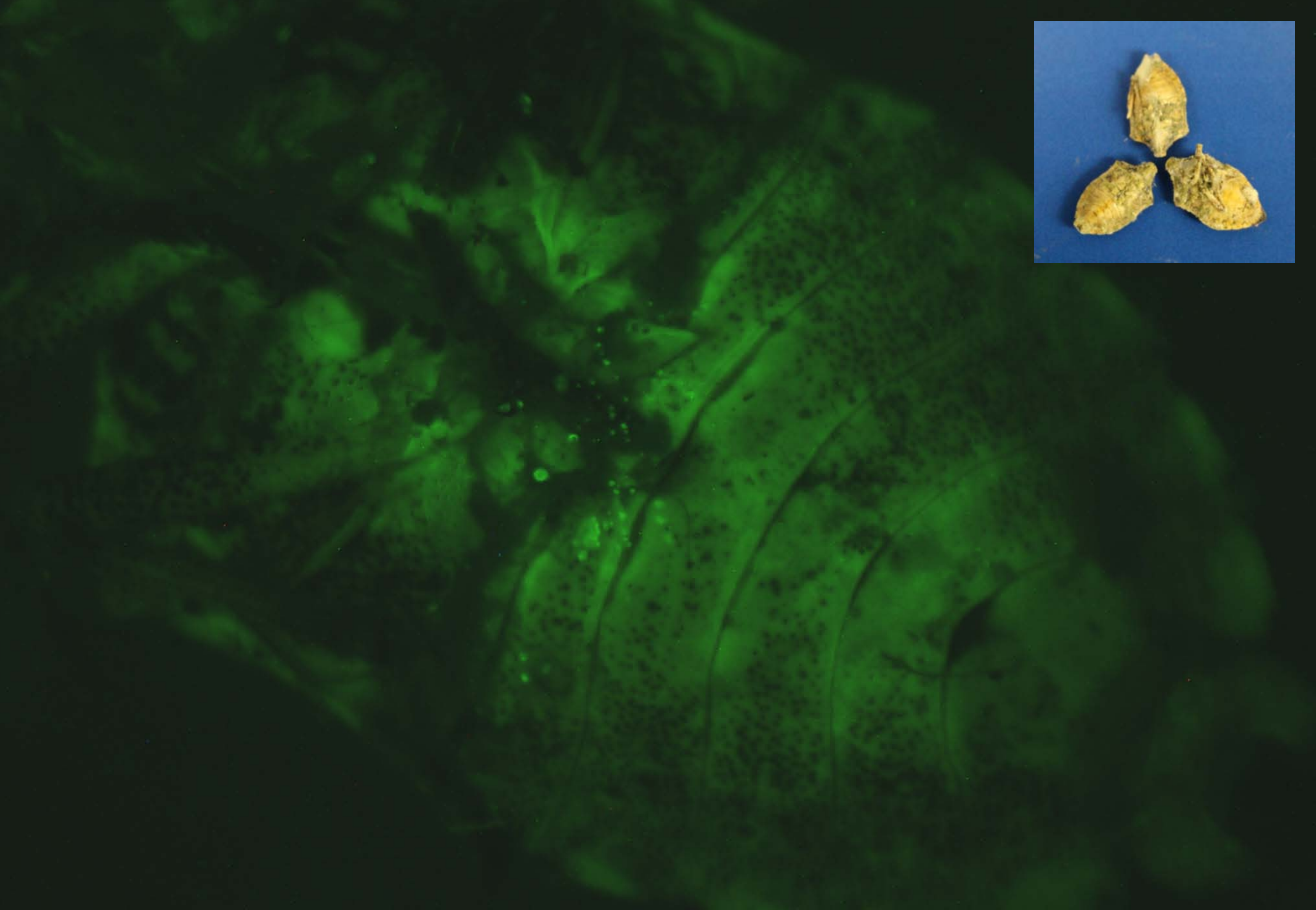
- The bacterial community on the cuticles of *Dalbulus maidis* and *Delphacodes kuscheli* inhibit *B. bassiana* (Toleda et al., 2011 J. Insect Sci 11, 29)
- *M. anisopliae* is inhibited by the bed bug defensive secretions (E)-2-hexenal and (E)-2-octenal (Ulrich et al 2015 Biocontrol 10.1007/s10526-015-9667-2)

Post-Penetration barriers

Cellular and humoral immunity

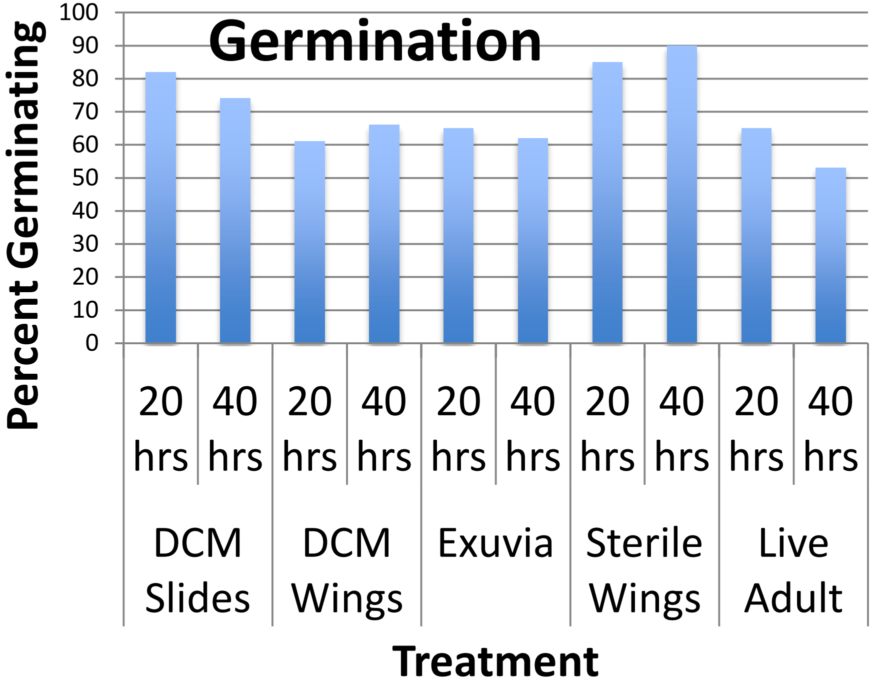
- Thanatin

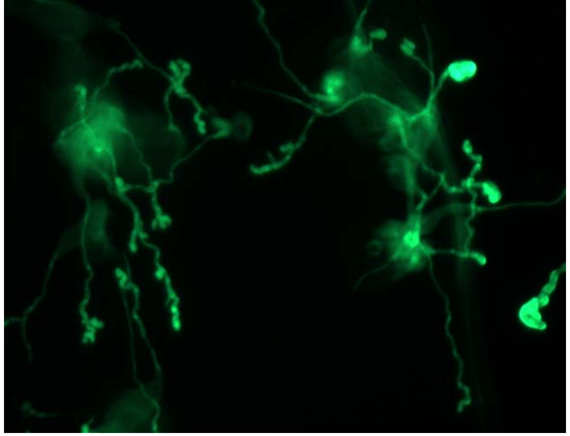
A “glow in the dark” BMSB produced by injecting 50 spores of Ma-GFP

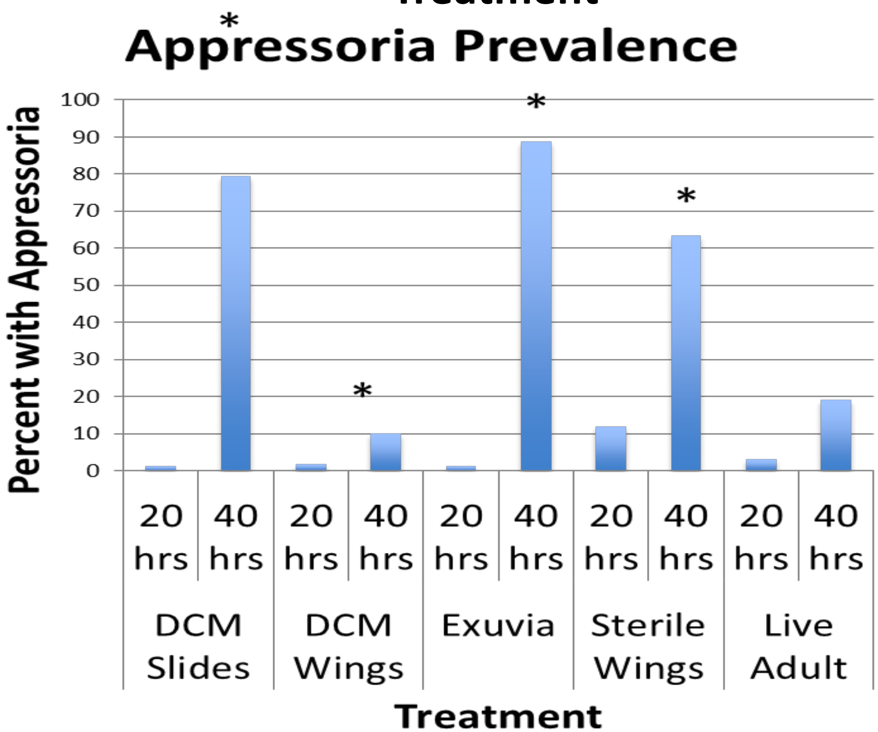


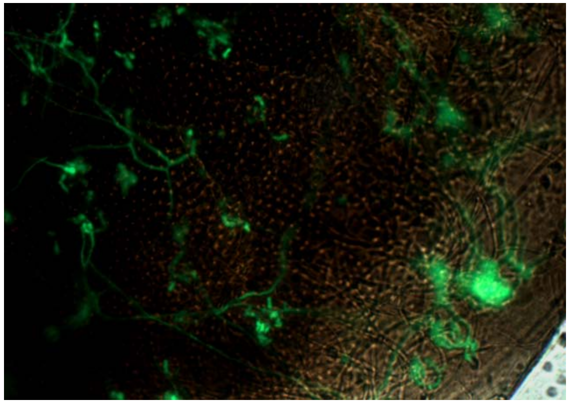
Mechanism behind low virulence of fungi against brown marmorated stink bug

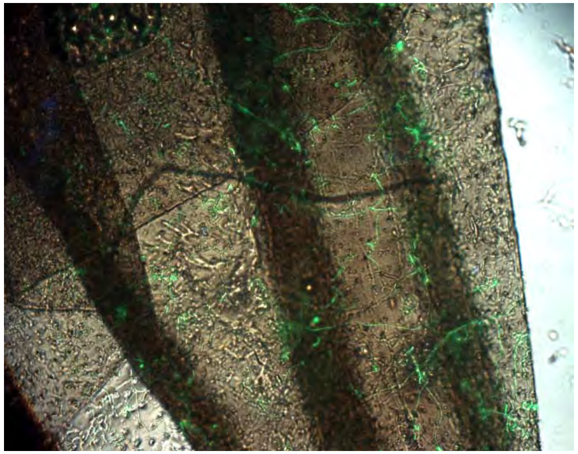
- **Brown marmorated stink bug defensive compounds may be the cause of poor fungal performance**
- **Two chemicals present in stink bug defensive secretions (trans-2-octenal and trans-2-decenal) found to strongly inhibit growth of entomopathogenic fungi**

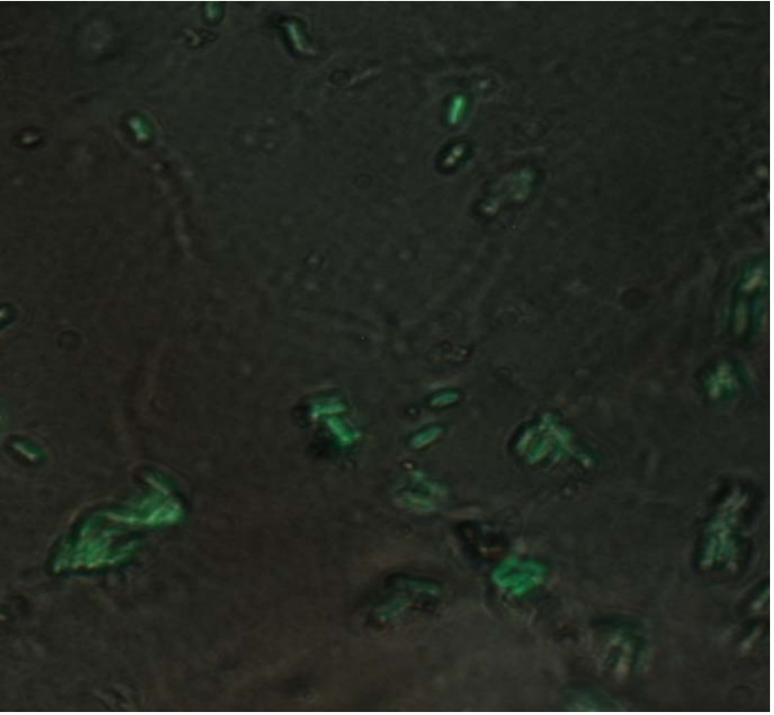

Germination
Percent Germinating
100
90
80
70
60
50
40
30
20
10
0
20 hrs
40 hrs
20 hrs
40 hrs
20 hrs
40 hrs
20 hrs
40 hrs
20 hrs
40 hrs
DCM Slides
DCM Wings
Exuvia
Sterile Wings
Live Adult
Treatment

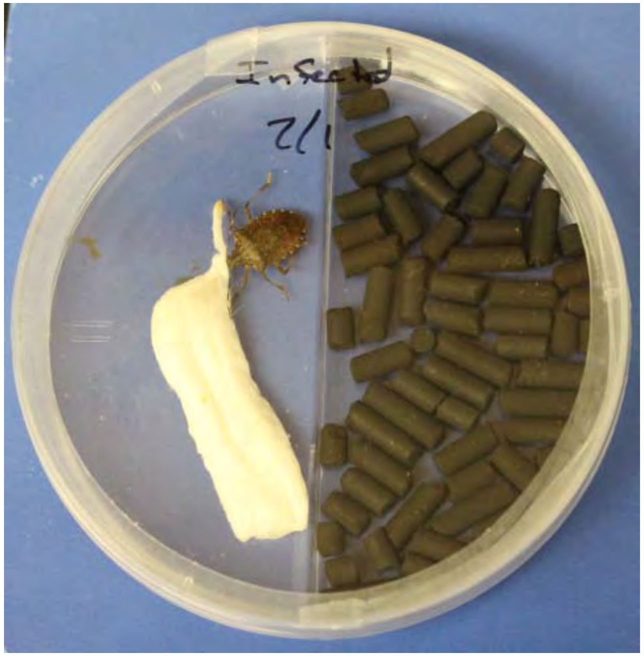



Appressoria Prevalence
Percent with Appressoria
100
90
80
70
60
50
40
30
20
10
0
*
*
*
*
20 hrs
40 hrs
20 hrs
40 hrs
20 hrs
40 hrs
20 hrs
40 hrs
20 hrs
40 hrs
DCM Slides
DCM Wings
Exuvia
Sterile Wings
Live Adult
Treatment

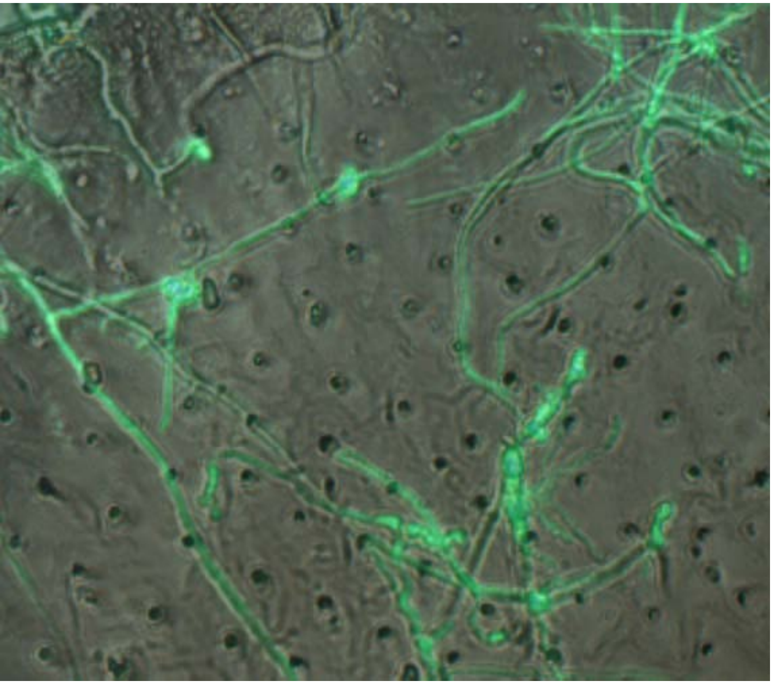






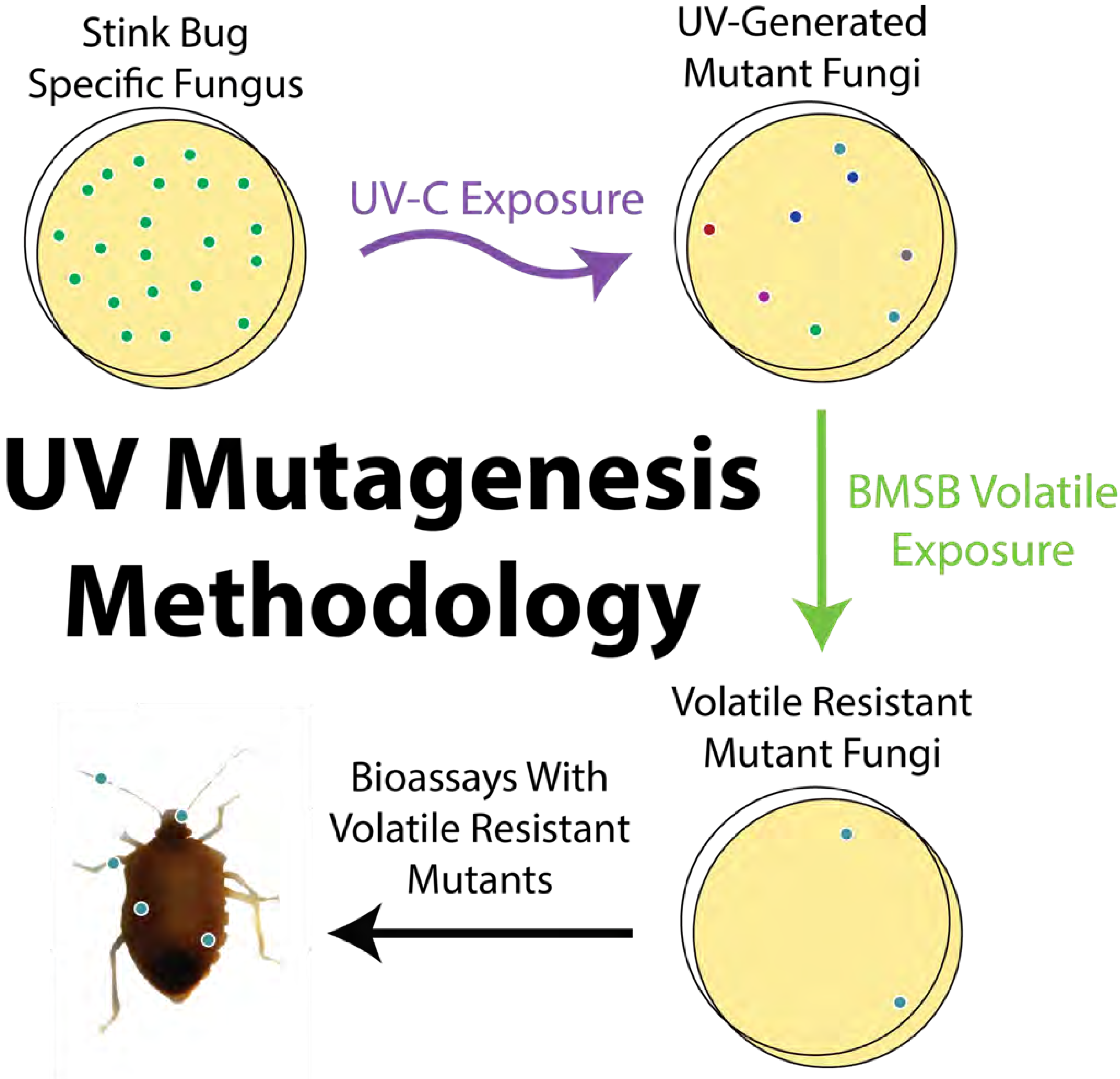


Low RH
No volatiles



High RH
Volatiles

- ***Metarhizium*** **infects and kills BMSB at high humidities.**
- **Poor performance of fungi at low humidities**
  - **Low humidities reduce germination**
  - **Increase susceptibility to volatiles**
- **BMSB relies heavily on volatiles; haemolymph defenses are weak.**


Stink Bug
Specific Fungus
UV-C Exposure
UV-Generated
Mutant Fungi
UV Mutagenesis
Methodology
BMSB Volatile
Exposure
Volatile Resistant
Mutant Fungi
Bioassays With
Volatile Resistant
Mutants

Resistant mutants have fluffy white growth-this could be because BMSB volatiles resemble chemicals fungi use for auto-inhibition of spores.

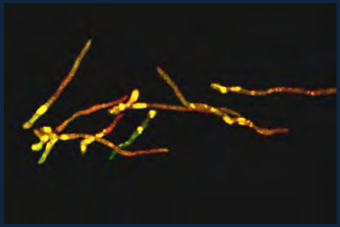
- -select for mutants that do not auto inhibit
- -transgenic approaches to alter regulation of sporulation genes

Conduct mass spec analysis of volatile production by infected BMSB

Determine role of microbial community on cuticle

Employ field cages to test laboratory results that single insects are more susceptible than clustered insects

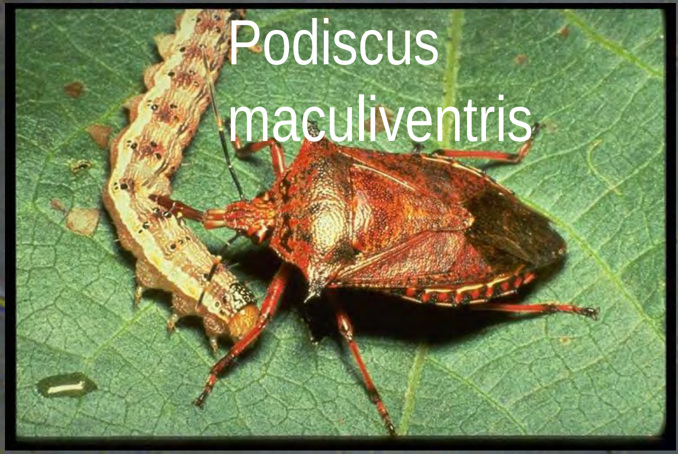
Protoplast fusion of BMSB-infecting *Metarhizium* strains with *M. acridum*.



Use GE to convert volatile and/or RH resistant strain to hypervirulence

Structure–activity analysis of thanatin, a 21-residue inducible insect defense peptide with sequence homology to frog skin antimicrobial peptides

Pascale Fehlbaum*†, Philippe Bulet*†‡, Serguey Chernysh§, Jean-Paul Briand¶, Jean-Pierre Roussel*, Lucienne Letellier‖, Charles Hetru*, and Jules A. Hoffmann*



1835 Agostino Bassi showed that a disease affecting silkworms was caused by a fungus - the first microorganism to be recognized as a contagious agent of animal disease.

Thanatin inhibits:
Beauveria
Some *Metarhizium*’s
Chalkbrood
(*Ascosphaera apis*)
and foulbrood
(*Paenibacillus larvae*)