Entomopathogenic fungi to suppress BMSB

Participants:
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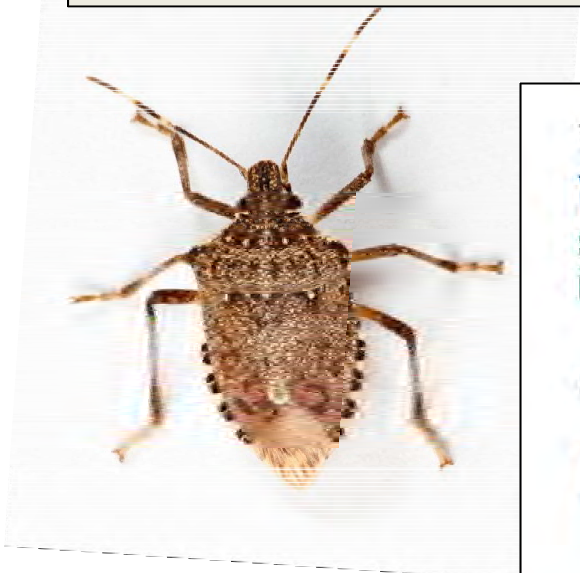
Department of Entomology, UMD



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Collaborating Institutions

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University of Maryland
Virginia Tech
OSU Oregon State University
NC State University

StopBMSB.org

Fungi to suppress BMSB

BMSB SCRI Grant (3 year, 2014)

Subobjective 2.2.2 - Identification of a single, well characterized strain of fungi expressing the optimum toxin combination for BMSB control

Subobjective 2.2.3 - Develop attract-and-kill and mass trapping strategies for management of BMSB in commercial crops

BMSB SCRI Renewal Grant

Subobjective 2.2.2 - Development of BMSB-Specific Fungal Entomopathogens (Ray St. Leger, Department of Entomology, UMD)

Entomopathogenic Fungi

- Fungi that colonize and kill their insect host
- Effective at controlling insects that won't readily consume topical pathogens
  - *Bacillus thuringiensis* (Bt)
- *Metarhizium anisopliae, Beauveria bassiana* and *Isaria fumosorosea* among the most common entomopathogenic fungi used
- Used in insect systems such as grasshoppers, flies, beetles, caterpillars and greenhouse pests

Objectives

- Determine the effects of entomopathogenic fungi on brown marmorated stink bug
  - Wild-type fungal strains
  - Additives (diatomaceous earth and horticultural oil)
  - Transgenic fungal strains
- Explore their potential as biological control agents

Wild-type Fungal Bioassay

- Strains evaluated:
  - *Metarhizium* (M): ARSEF 1548, ARSEF 2547, ARSEF 1055, F52 (4 strains)
  - *Beauveria* (B): GHA, Botanigard (2 strains)
  - *Isaria* (I): ARSEF 3581 (1 strain)
  - Other (U): ARSEF 10386, Unidentified fungus (isolated from lab colony) (2 strains)

Wild-type Fungal Bioassay Methods

- Fungi plated on PDA media
- Spore suspensions of $1x10^7$ conidia/mL used with .01% Tween (later DI water)
- BMSB submerged in suspension, placed in plastic bowls with food and water
- Stink bugs monitored for mortality
- Tested on nymphs and adults
- Not all treatments represented in each bioassay (9 bioassays)

Wild-Type Bioassays

		Mean % Mortality			
		x̄ % Mortality (Day 3)		x̄ % Mortality (Day 7)	
Bioassay #	Treatment	Adults	Nymphs	Adults	Nymphs
1	1548 (M)	0	3.33 (±3.33)	10 (±4.47)	30 (±4.47)a
1	2547 (M)	3.33 (±3.33)	0	13.33 (±6.67)	43.33 (±9.54)a
1	Tween	3.33 (±3.33)	0	3.33 (±3.33)	0b
1	Water	3.33 (±3.33)	0	10 (±4.47)	0b
2	1055 (M)	10 (±10)	46.67 (±15.20)	36.67 (±12.01)	100 (±0)
2	GHA - Botanigard (B)	3.33 (±3.33)	40 (±7.30)	30 (±11.25)	90 (±6.83)
2	Tween	10 (±6.83)	40 (±13.66)	40 (±15.49)	80 (±7.30)
2	Water	6.67 (±4.21)	26.67 (±8.43)	33.33 (±9.88)	73.33 (±9.88)
3	2575 (M)	3.33 (±3.33)	40 (±7.30)	43.33 (±8.02)	93.33 (±4.21)
3	Unidentified Fungus (U)	10 (±6.83)	20 (±7.30)	56.67 (±9.54)	93.33 (±4.21)
3	Tween	10 (±4.47)	16.67 (±6.14)	66.67 (±8.43)	86.67 (±6.67)
3	Water	0	16.67 (±8.02)	33.33 (±8.43)	83.33 (±6.14)
4	F52 (M)	25 (±5.00)	25 (±12.58)	60 (±8.16)	70 (±12.91)
4	GHA - USDA (B)	30 (±12.91)	20 (±14.14)	65 (±9.57)	65 (±9.57)
4	3581 (I)	20 (±8.16)	40 (±14.14)	55 (±5.00)	80 (±0)
4	Water	25 (±18.93)	35 (±9.57)	45 (±18.93)	65 (±5.00)
5	10386 (U)	-	13.33 (±6.67)b	-	33.33 (±8.43)b
5	3581 (I)	-	30 (±8.56)a	-	73.33 (±8.43)a
5	Tween	-	23.33 (±3.33)b	-	33.33 (±6.67)b
5	Water	-	3.33 (±3.33)b	-	13.33 (±6.67)b

Wild-Type Bioassays

Bioassay #	Treatment	Mean % Mortality			
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5	3581 (I)	-	30 (±8.56)a	-	73.33 (±8.43)a
5	Tween	-	23.33 (±3.33)b	-	33.33 (±6.67)b
5	Water	-	3.33 (±3.33)b	-	13.33 (±6.67)b

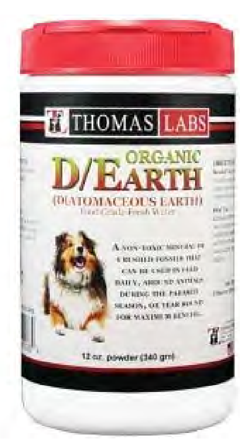
Wild-Type Fungal Bioassays with Additives

- Diatomaceous earth (DE) and horticultural oil tested in conjunction with F52 (M), GHA (B) and 3581 (I)

- Testing for increased mortality with additives

- 50g/L DE, 7% oil used in treatments

- All other procedures as before



Wild-Type Bioassays with Additives

Treatment	Mortality	
	x̄ % Mortality (Day 3)	x̄ % Mortality (Day 7)
Water	5.00 (±5.00)	30.00 (±12.91)
DE	20.00 (±8.16)	35.00 (±9.57)
DE/Oil	40.00 (±11.54)*	60.00 (±8.16)
Oil	40.00 (±8.16)*	55.00 (±12.58)
3581 (I)	20.00 (±0)	45.00 (±9.57)
3581/DE	5.00 (±5.00)	40.00 (±14.14)
3581/DE/Oil	20.00 (±0)	50.00 (±10.00)
3581/Oil	50.00 (±10.00)*	65.00 (±9.57)
F52 (M)	5.00 (±5.00)	35.00 (±5.00)
F52/DE	0 (±0)	50.00 (±10.00)
F52/DE/Oil	10.00 (±10.00)	65.00 (±12.58)
F52/Oil	40.00 (±21.60)*	70.00 (±10.00)
GHA (B)	5.00 (±5.00)	10.00 (±5.77)
GHA/DE	0 (±0)	25.00 (±15.00)
GHA/DE/Oil	20.00 (±8.16)	35.00 (±9.57)
GHA/Oil	15.00 (±5.00)	30.00 (±10.00)

Wild-Type Bioassays with Additives

Treatment	Mortality	
	x̄ % Mortality (Day 3)	x̄ % Mortality (Day 7)
Water	5.00 (±5.00)	30.00 (±12.91)
DE	20.00 (±8.16)	35.00 (±9.57)
DE/Oil	40.00 (±11.54)*	60.00 (±8.16)
Oil	40.00 (±8.16)*	55.00 (±12.58)
3581 (I)	20.00 (±0)	45.00 (±9.57)
3581/DE	5.00 (±5.00)	40.00 (±14.14)
3581/DE/Oil	20.00 (±0)	50.00 (±10.00)
3581/Oil	50.00 (±10.00)*	65.00 (±9.57)
F52 (M)	5.00 (±5.00)	35.00 (±5.00)
F52/DE	0 (±0)	50.00 (±10.00)
F52/DE/Oil	10.00 (±10.00)	65.00 (±12.58)
F52/Oil	40.00 (±21.60)*	70.00 (±10.00)
GHA (B)	5.00 (±5.00)	10.00 (±5.77)
GHA/DE	0 (±0)	25.00 (±15.00)
GHA/DE/Oil	20.00 (±8.16)	35.00 (±9.57)
GHA/Oil	15.00 (±5.00)	30.00 (±10.00)

Transgenic Fungal Bioassays

- Use of transgenic fungi successful in other insect systems
  - Scorpion and spider toxins
- Fungi engineered to express spider neuropeptides
- *Metarhizium* strains tested: Hv1a-1548, Dc1a-1548, As1a-1548, Ta1a-1548
- All other procedures as before

Transgenic Bioassays

Treatment	Mortality		Fungal Growth	
	$\bar{x}$ % Mortality (Day 3)	$\bar{x}$ % Mortality (Day 7)	$\bar{x}$ Days to Growth	$\bar{x}$ % dead with Growth
1548 WT	20 (±14.14)	95 (±5.00)	4 (±0.87)	40 (±8.16)
As1a	10 (±5.77)	80 (±14.14)	2 (±0)	20 (±8.16)
Dc1a	15 (±9.57)	70 (±10.00)	2.67 (±1.11)	40 (±8.16)
Hv1a	30 (±10.00)	65 (±5.00)	3.25 (±1.43)	40 (±8.16)
Ta1a	20 (±8.16)	75 (±9.57)	8 (±0)	10 (±5.77)
Water	20 (±8.16)	60 (±14.14)	-	-

Summary

- Overall levels of mortality low
- Little difference seen between wild-type strains (with and without additives) and transgenic strains
- Low virulence of fungi
- No indication that any of the entomopathogenic fungi evaluated would be effective as a biological control ☹

Mechanism behind low virulence of fungi against brown marmorated stink bug

- Many insects protect themselves from fungal infection via chemical defense
  - Earwigs
  - Sawflies
  - Bed bugs
  - Pentatomids
- Brown marmorated stink bug defensive compounds may be the cause of poor fungal performance

Objectives

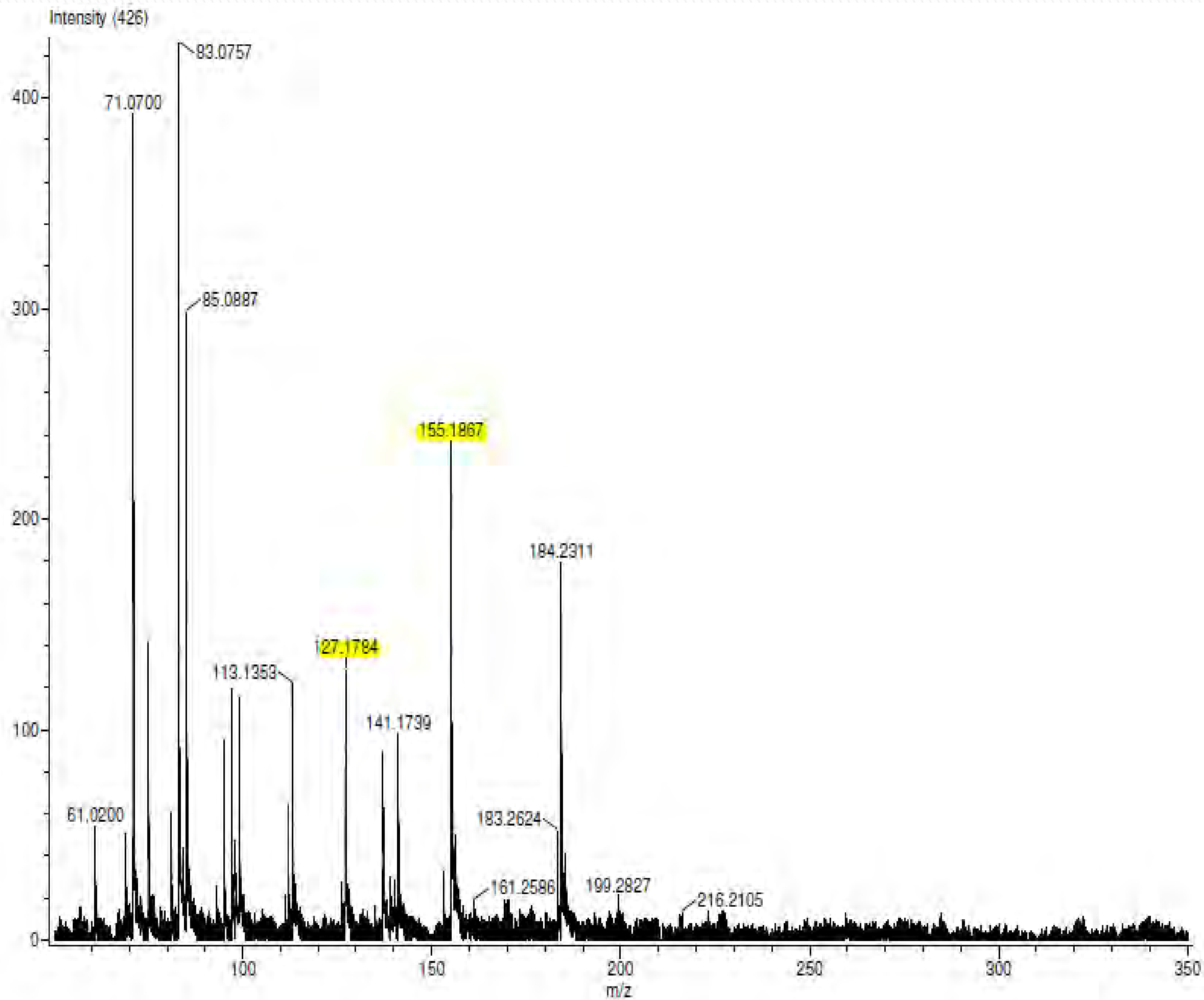
- Identify constituents of brown marmorated stink bug defensive compound

- Evaluate effects of defensive compounds against entomopathogenic fungi
  - Fungal growth
  - Spore germination

Defensive compounds

- From literature and other studies
  - Too many chemicals to ID, based search on *a priori* hypothesis
  - Predict that trans-2-octenal and trans-2-decenal are potential candidates

- Analyze brown marmorated stink bug secretions to confirm presence of trans-2-octenal and trans-2-decenal
  - AccuTOF mass spectrometer equipped with confined Direct Analysis in Real Time (cDART) ion source



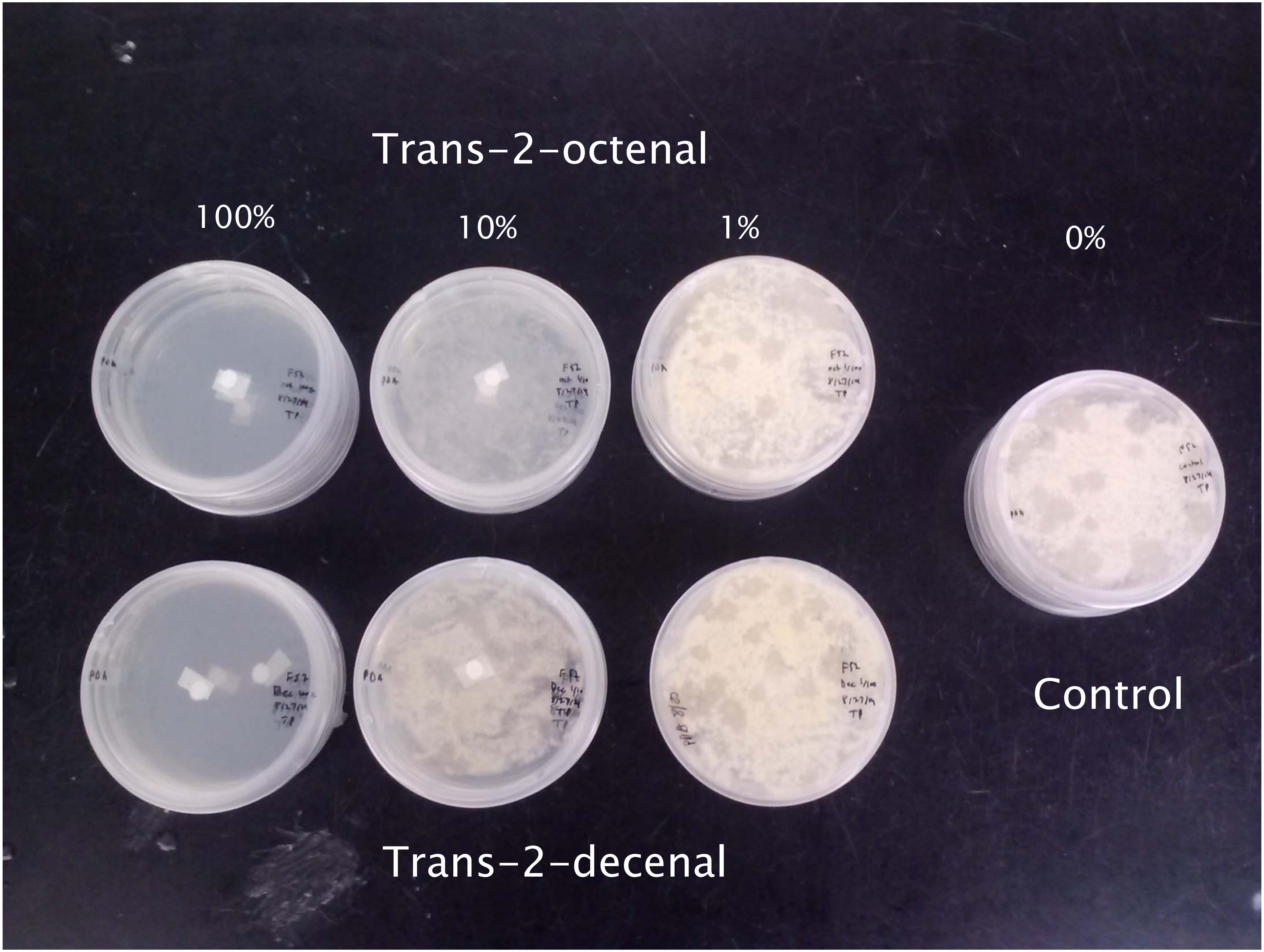
Intensity (426)
83.0757
71.0700
85.0887
155.1867
184.2311
127.1784
113.1353
141.1739
61.0200
183.2624
161.2586
199.2827
216.2105
400
300
200
100
0
100
150
200
250
300
350
m/z

Defensive compound effect on fungal growth – Inhibition

- 3 fungi evaluated
  - F52 (*Metarhizium*), GHA (*Beauveria*), ARSEF 3581 (*Isaria*)
- Fungi plated on PDA media
- 5 µL trans-2-octenal or trans-2-decenal applied to filter paper disc on inside lid of petri dish
  - 100%, 10%, 1%, 0% concentrations

Defensive compound effect on fungal growth – Inhibition

- Inhibition of fungi
  - Fungi grown for 3 days at 27^{o} C
  - # of plates with / without fungal growth recorded


Trans-2-octenal
100%
10%
1%
0%
Control
Trans-2-decenal

Fungus	Mean % Petri dishes without Fungal Growth (Inhibition)						
	Trans-2-octenal Concentration			Trans-2-decenal Concentration			
	100%	10%	1%	100%	10%	1%	Control
F52 (M)	100% (±0)	66% (±33.33)	0 (±0)	100% (±0)	33% (±33.33)	0 (±0)	0 (±0)
GHA (B)	100% (±0)	100% (±0)	0 (±0)	100% (±0)	66% (±33.33)	0 (±0)	0 (±0)
3581 (I)	100% (±0)	0 (±0)	0 (±0)	100% (±0)	33% (±33.33)	0 (±0)	0 (±0)

- Complete inhibition of fungal growth at 100% concentration
- No inhibition at 1% or 0% concentrations
- Partial inhibition at 10% concentration for both chemicals, and all fungal strains

Fungus	Mean % Petri dishes without Fungal Growth (Inhibition)						
	Trans-2-octenal Concentration			Trans-2-decenal Concentration			
	100%	10%	1%	100%	10%	1%	Control
F52 (M)	100% (±0)	66% (±33.33)	0 (±0)	100% (±0)	33% (±33.33)	0 (±0)	0 (±0)
GHA (B)	100% (±0)	100% (±0)	0 (±0)	100% (±0)	66% (±33.33)	0 (±0)	0 (±0)
3581 (I)	100% (±0)	0 (±0)	0 (±0)	100% (±0)	33% (±33.33)	0 (±0)	0 (±0)

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Fungus	Mean % Petri dishes without Fungal Growth (Inhibition)						
	Trans-2-octenal Concentration			Trans-2-decenal Concentration			
	100%	10%	1%	100%	10%	1%	Control
F52 (M)	100% (±0)	66% (±33.33)	0 (±0)	100% (±0)	33% (±33.33)	0 (±0)	0 (±0)
GHA (B)	100% (±0)	100% (±0)	0 (±0)	100% (±0)	66% (±33.33)	0 (±0)	0 (±0)
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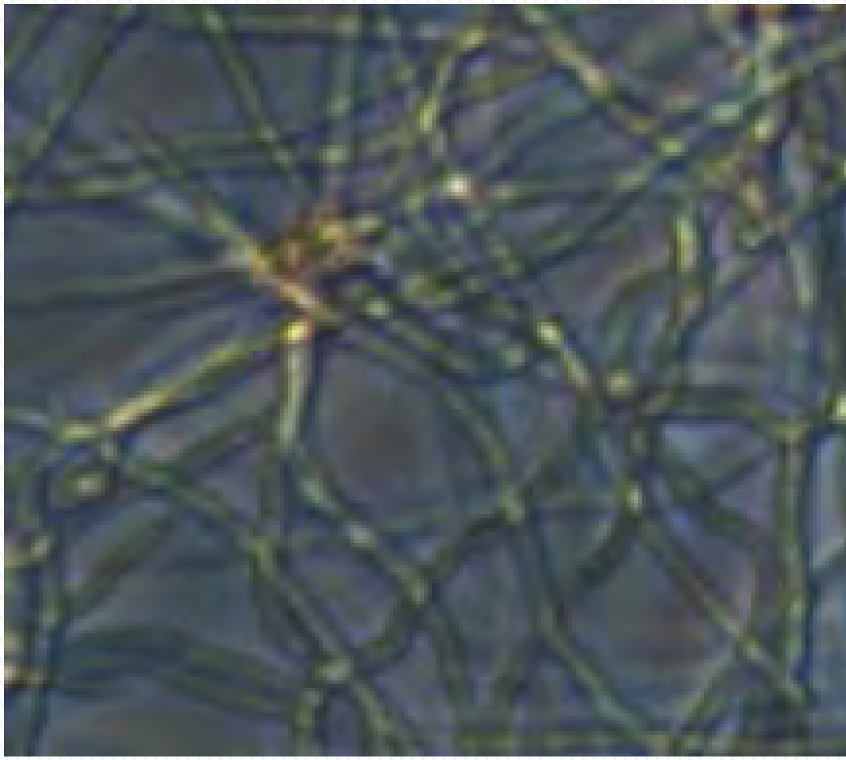
3581 (I)	100% (±0)	0 (±0)	0 (±0)	100% (±0)	33% (±33.33)	0 (±0)	0 (±0)

- Complete inhibition of fungal growth at 100% concentration
- No inhibition at 1% or 0% concentrations
- Partial inhibition at 10% concentration for both chemicals, and all fungal strains

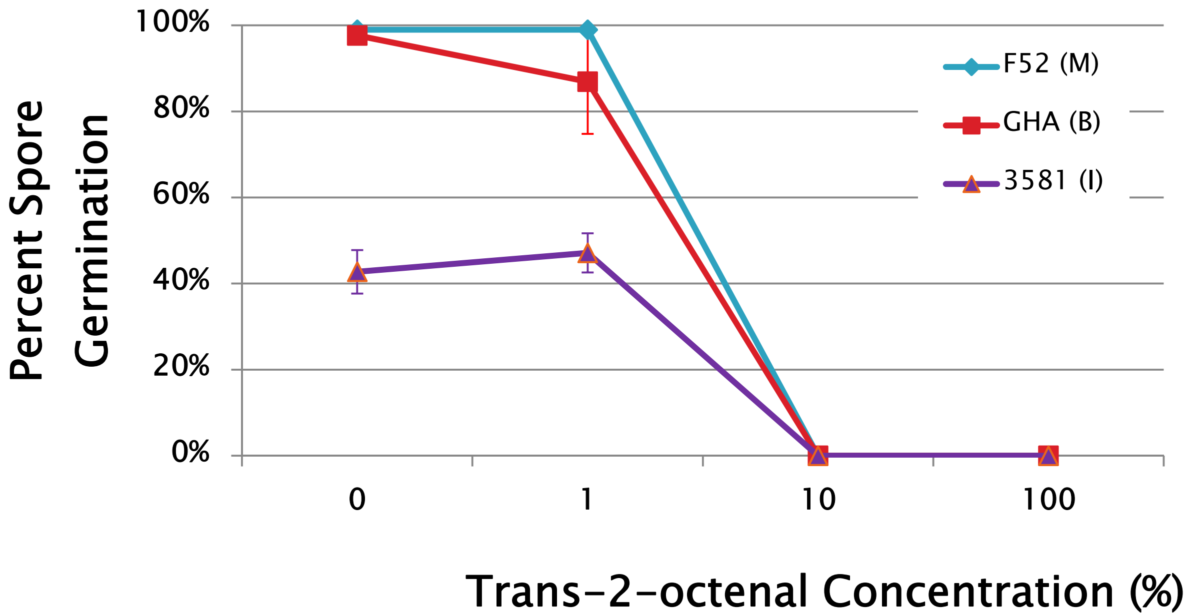
Defensive compound effect on spore germination

- 2 mL liquid PDB media containing spore suspension added to 35 mm petri dishes
- Trans-2-octenal and trans-2-decenal added at same concentrations as previous experiment
- Dishes photographed at 400x magnification 1 day after treatment, % spore germination calculated
- Spore germination defined as presence of germ tube

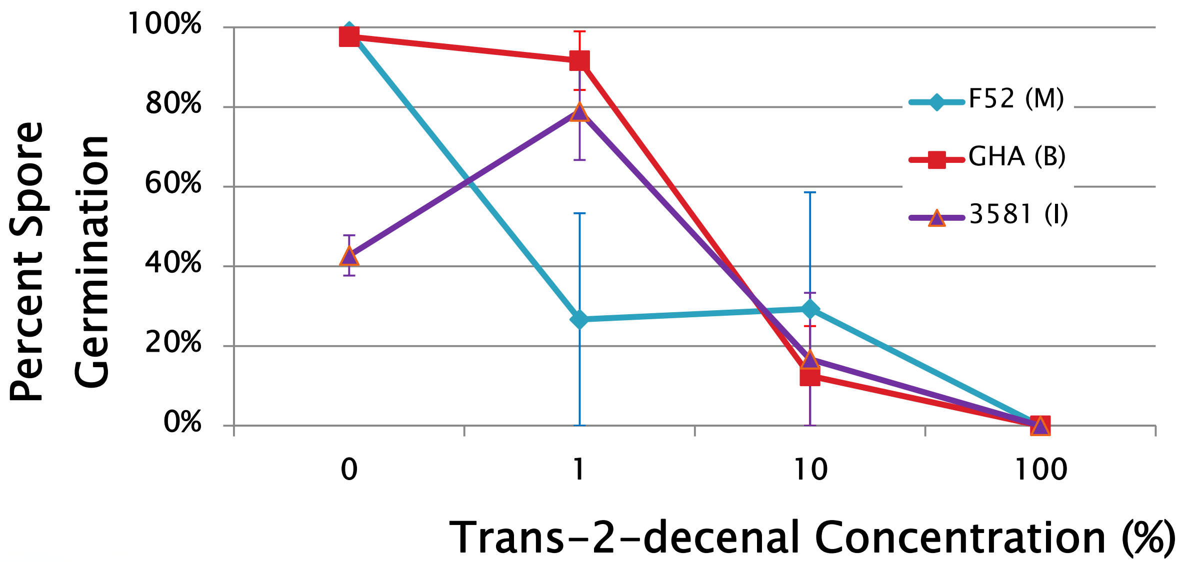


Germinated F52

Ungerminated F52



100%
80%
60%
40%
20%
0%
Percent Spore Germination
0
1
10
100
F52 (M)
GHA (B)
3581 (I)
Trans-2-octenal Concentration (%)



100%
80%
60%
40%
20%
0%
Percent Spore Germination
0
1
10
100
F52 (M)
GHA (B)
3581 (I)
Trans-2-decenal Concentration (%)

Summary

- Trans-2-octenal and trans-2-decenal present in live brown marmorated stink bug defensive secretions

- Both chemicals inhibit fungal growth and spore germination in laboratory setting

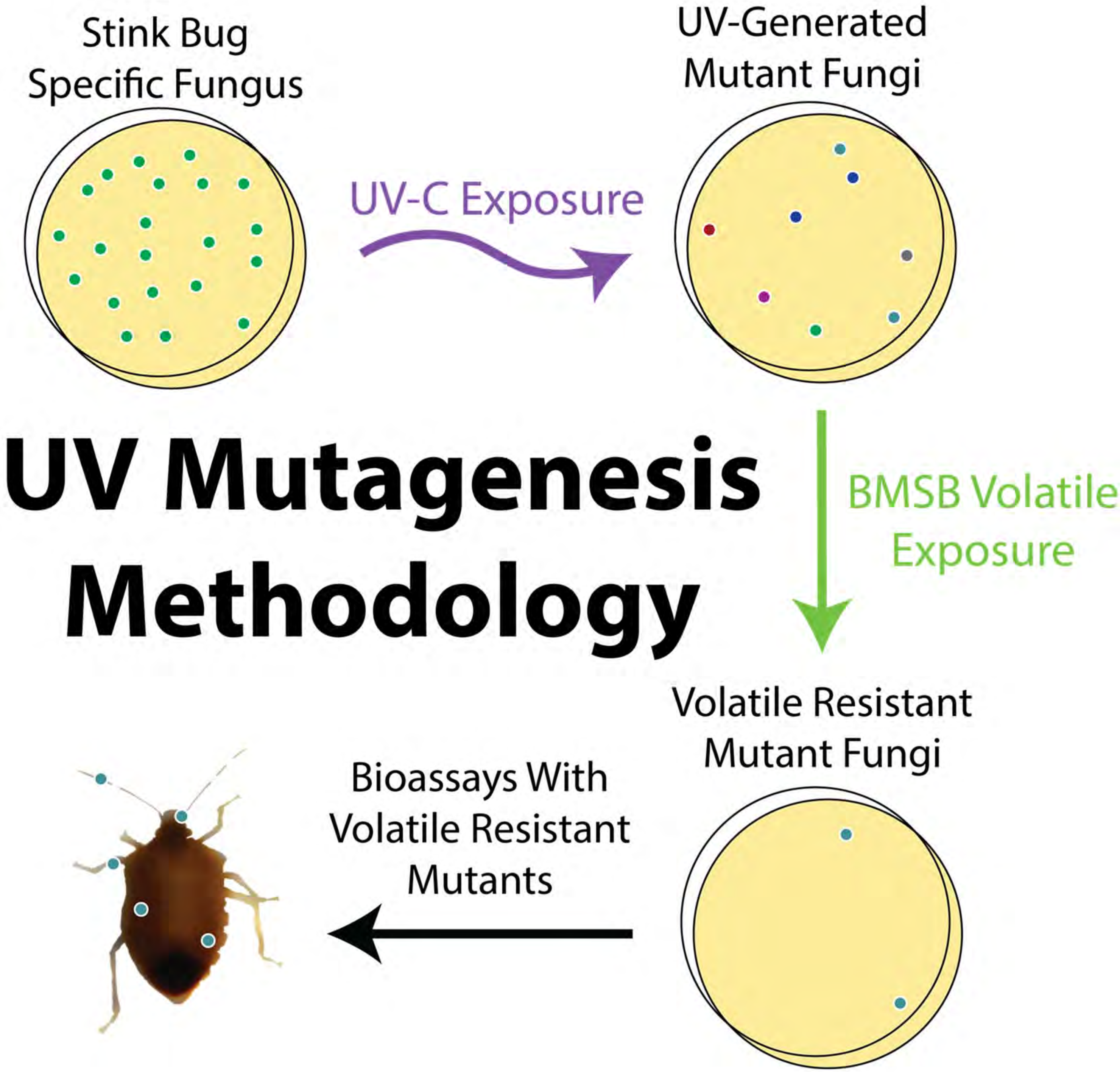
Conclusions

- Additional challenges with using entomopathogenic fungi as biological control
  - Low and inconsistent mortality
  - Additives and transgenic strains do not improve efficacy

- Defensive chemicals may explain low virulence of fungi against brown marmorated stink bug
  - Inhibition of fungal growth/spore germination

Creating a pseudo-wild type strain

- Inducing mutations in wild-type strains with the hopes of getting a beneficial mutation
- Attempt to induce mutation that confers resistance to stink bug defensive compounds
- Fewer obstacles to use than with GMO products


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

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