

2.2.3 Attract-and-Kill, 2.2.4 Repellents, 2.2.7 Cultural Techniques for IPM, 2.2.8 RNAi



Funding



United States
Department of
Agriculture

National Institute
of Food and
Agriculture

Specialty Crop Research Initiative
Grant #2011-01413-30937

Collaborating Institutions



Cornell University



Virginia Tech



2.2.3 Develop attract-and-kill strategies for controlling BMSB



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Behavioral Basis of AK

- Baseline questions to be addressed:
 - 1) How large is the area of arrestment of BMSB around a pheromone source?
 - 2) How long can we retain BMSB adults on baited vs. non-baited crop hosts?
 - 3) Can we precisely target management to obtain a large kill of BMSB throughout the season?



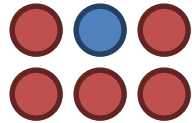
Behavioral Basis of AK

- Baseline questions to be addressed:
 - 1) How large is the area of arrestment of BMSB around a pheromone source? **<2.5 m**
 - 2) How long can we retain BMSB adults on baited vs. non-baited crop hosts? **~ 24 hrs**
 - 3) Can we precisely target management to obtain a large kill of BMSB throughout the season?

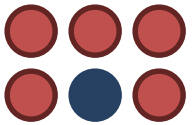


Methods: Attract & Kill

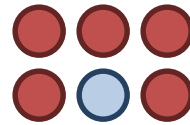
100 mg + MDT



1000 mg + MDT



Control



x 4 Blocks

Sprayed every 7 d

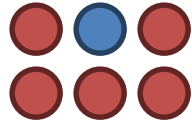
Sampled @ 1 & 6 d

16 weeks

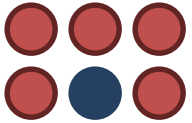


Methods: Attract & Kill

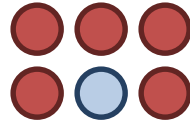
100 mg + MDT



1000 mg + MDT



Control



x 4 Blocks

Sprayed every 7 d

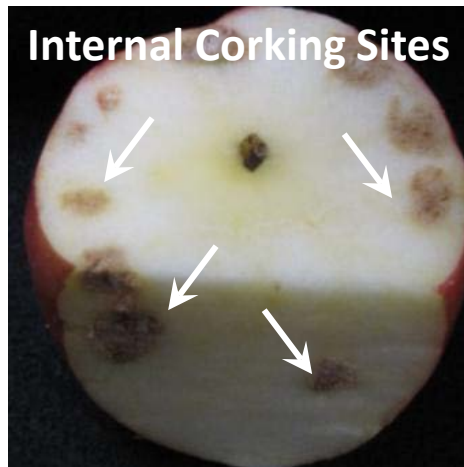
Sampled @ 1 & 6 d

16 weeks

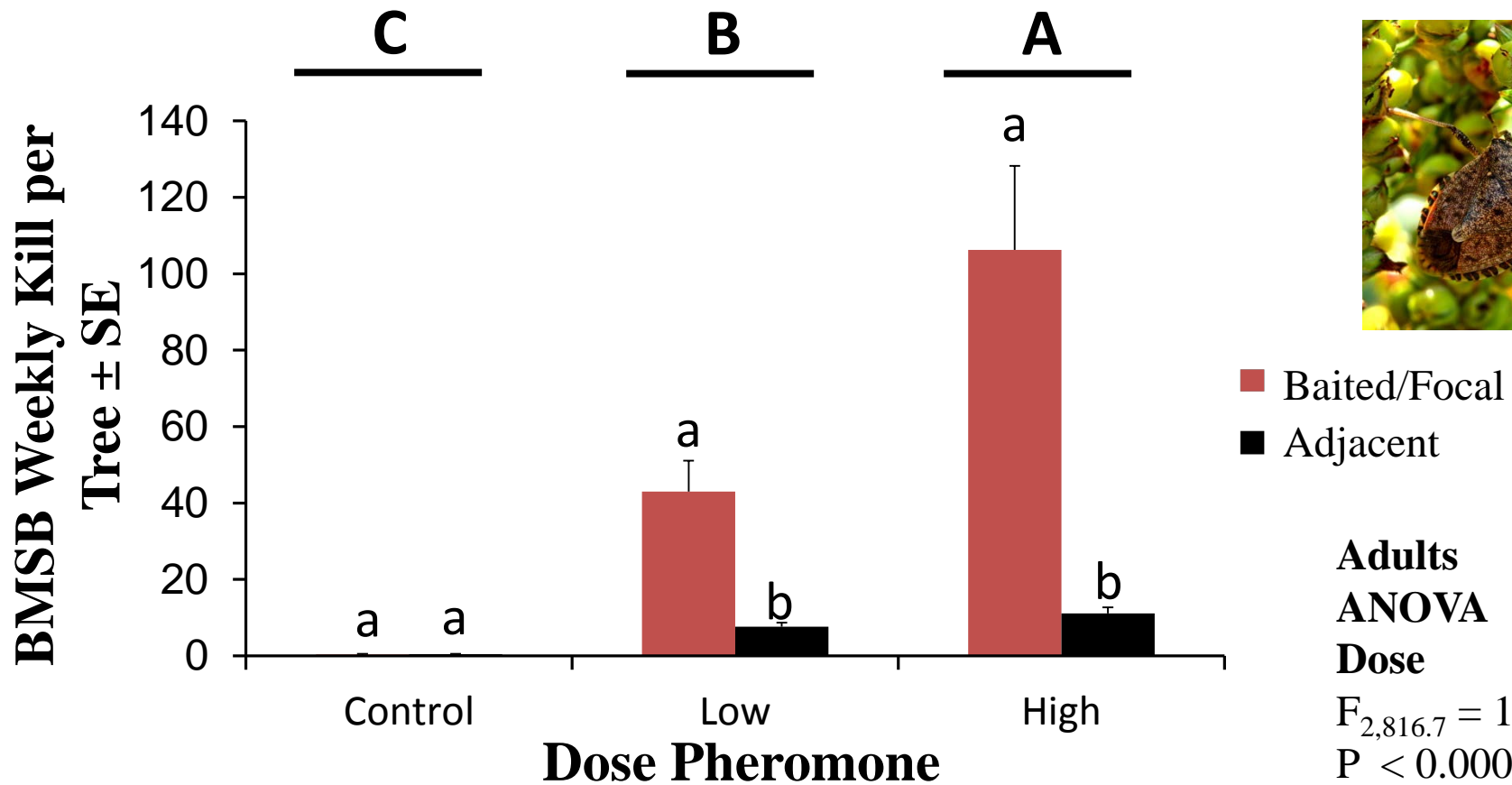


Methods: Attract & Kill

Harvest sample at end of season
From **baited & adjacent** trees
50 fruit per tree



Adults: Efficiency of Kill Sites & Spillover



**Adults
ANOVA**

Dose

$F_{2,816.7} = 1091.3$

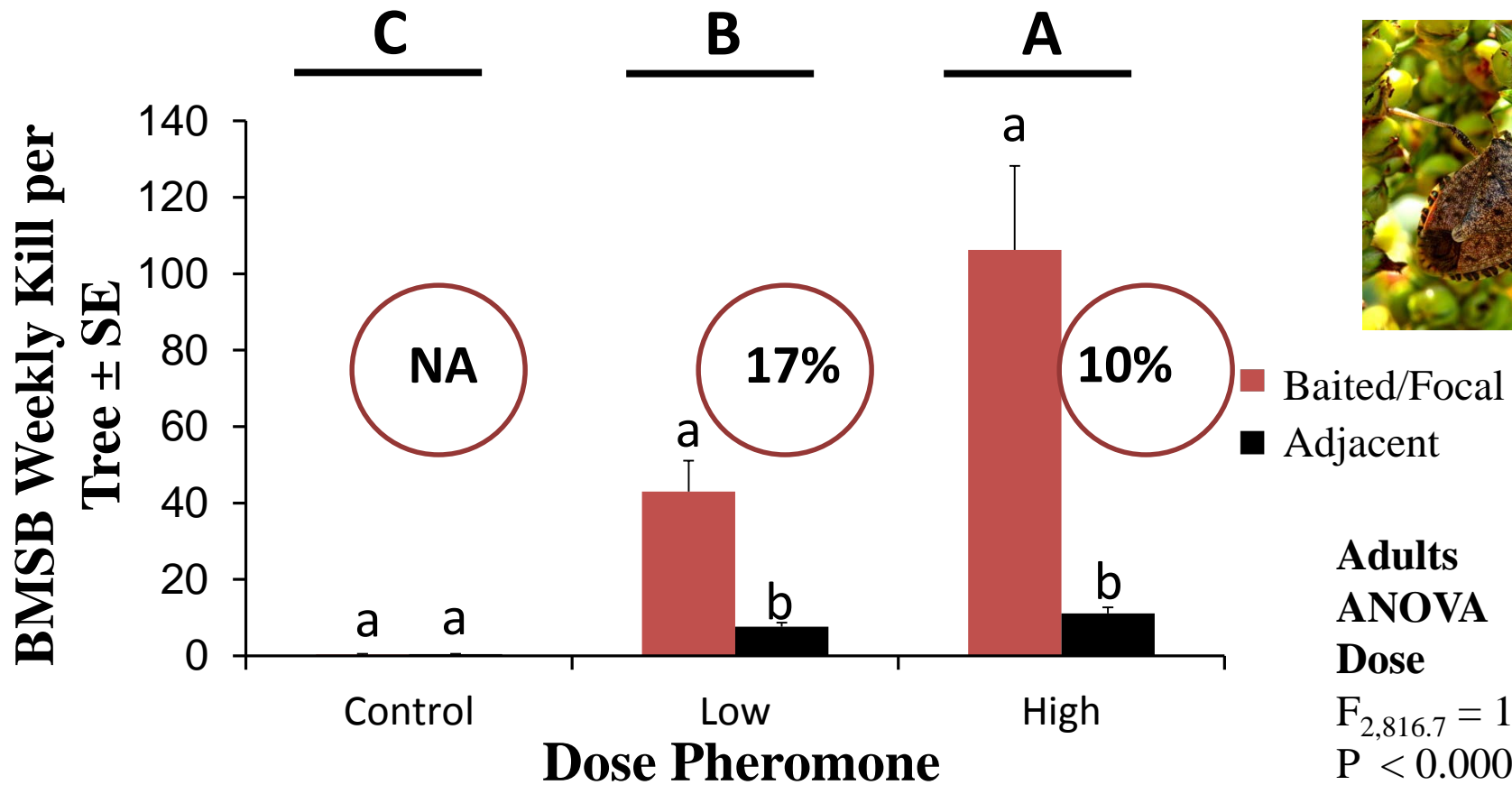
$P < 0.0001$

Focal Tree

$F_{1,816.5} = 401.8$

$P < 0.0001$

Adults: Efficiency of Kill Sites & Spillover



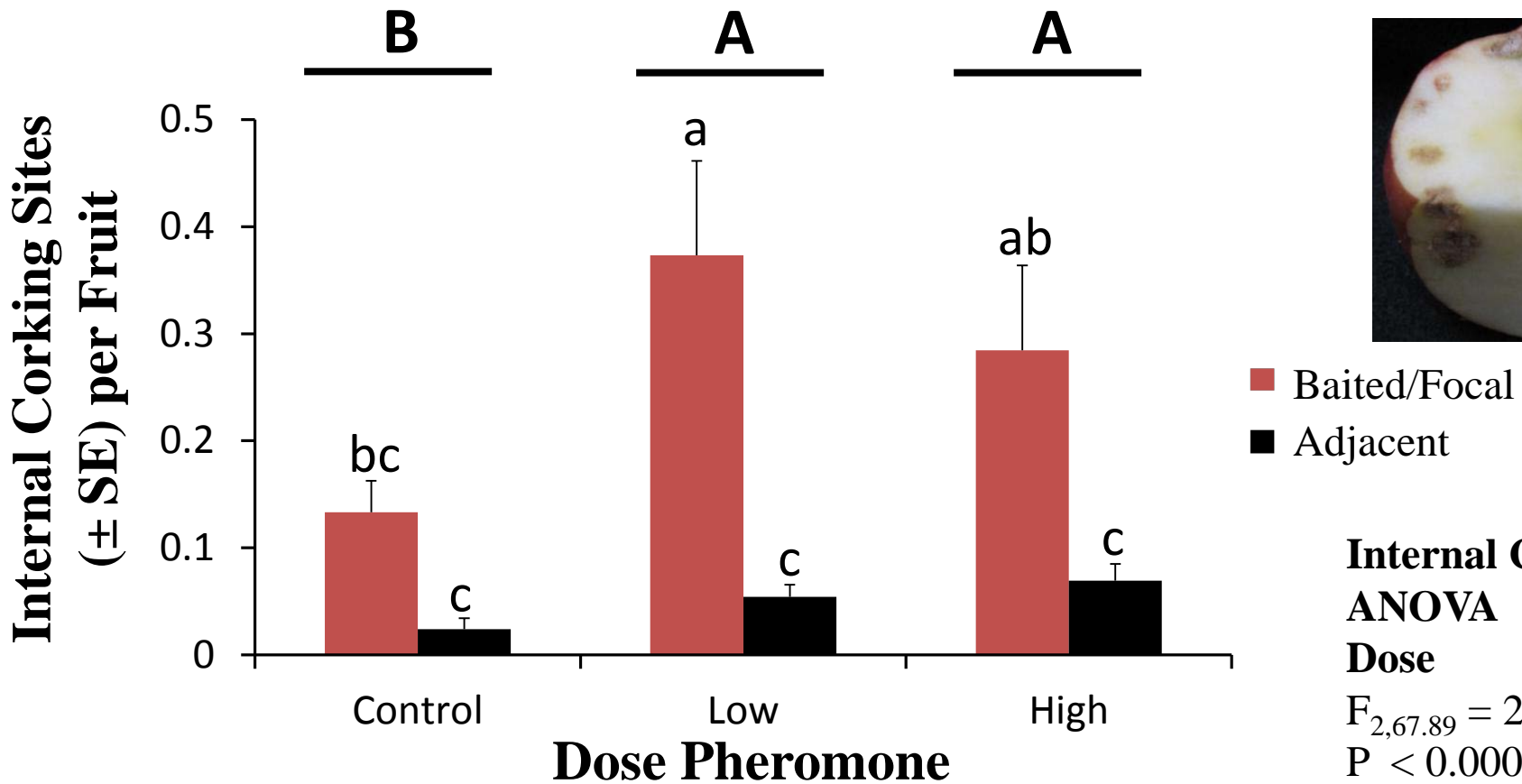
Adults
ANOVA
Dose

$F_{2,816.7} = 1091.3$
 $P < 0.0001$

Focal Tree

$F_{1,816.5} = 401.8$
 $P < 0.0001$

Damage: Internal Corking



Internal Corking ANOVA

Dose

$$F_{2,67.89} = 226.2$$

$$P < 0.0001$$

Focal Tree

$$F_{1,1255} = 4580$$

$$P < 0.0001$$

SPLAT Attract-and-Kill Technology



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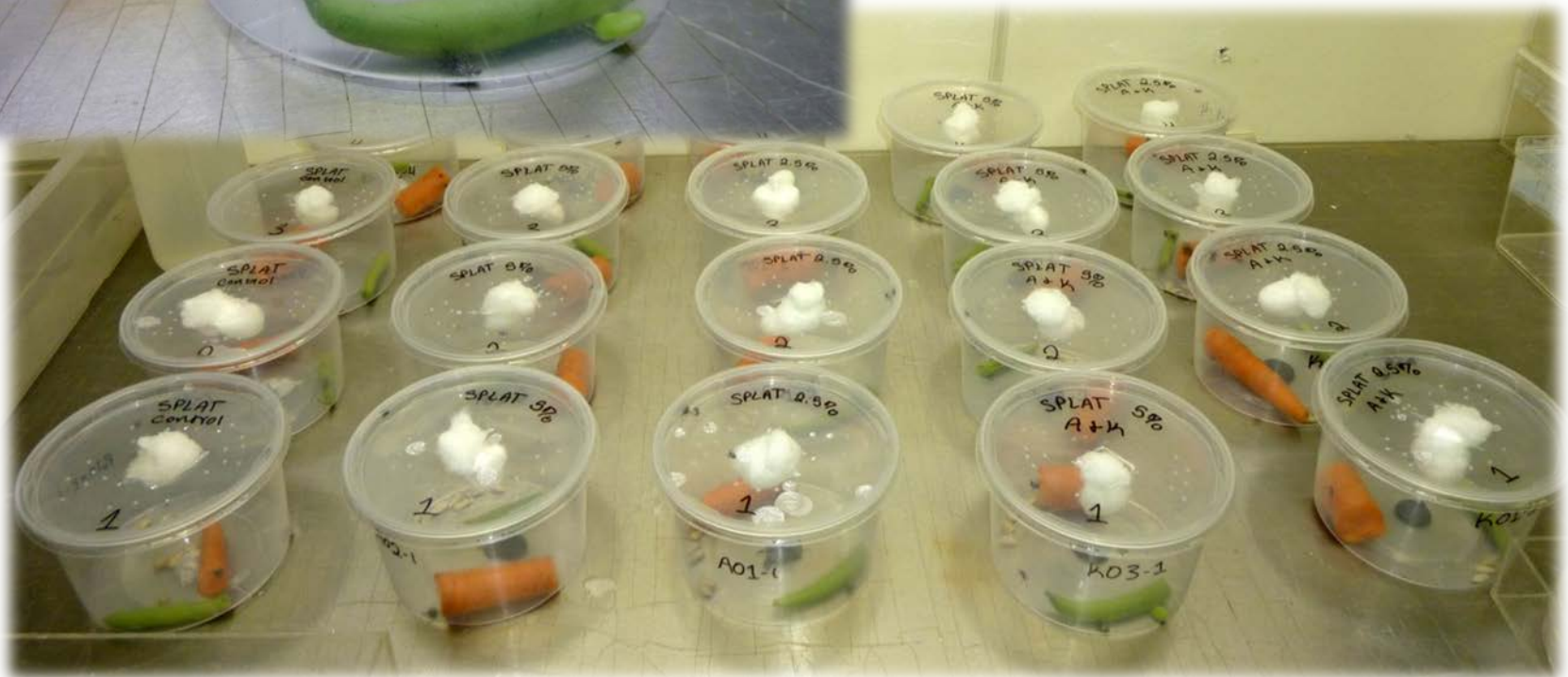


Virginia Tech



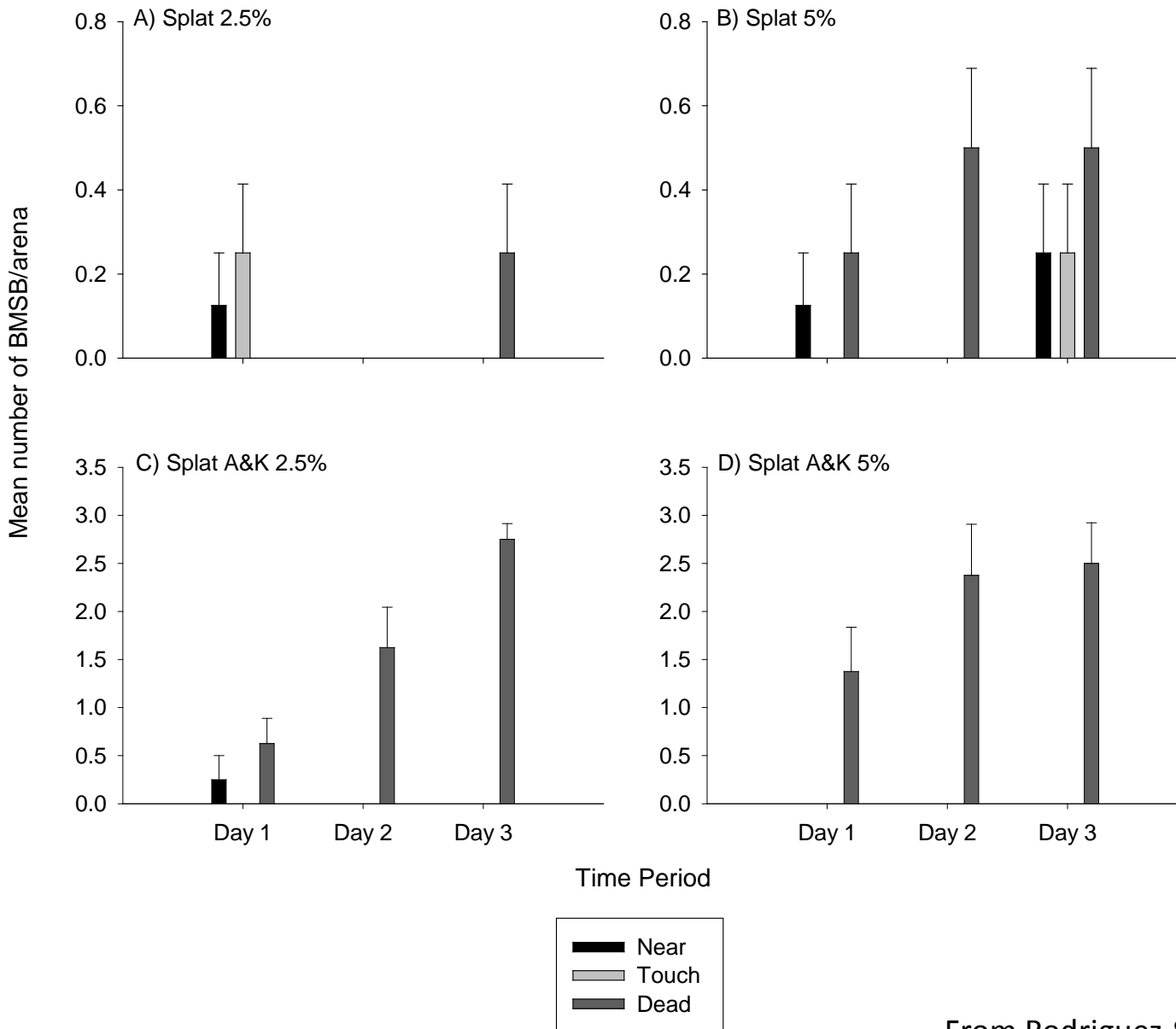
Methods IV. SPLAT Bioassays

- Treatments
 - SPLAT for attraction of BMSB (2.5%)
 - SPLAT for attraction of BMSB (5%)
 - SPLAT for A&K of BMSB (2.5%)
 - SPLAT for A&K of BMSB (5%)
 - SPLAT control
- 1 g dollop per arena
- 5 3rd or 4th instar nymphs
- 4 replications/treatment
- Recorded
 - mortality every 6 and 12 hours for 3 days
- All arenas contained green beans, carrots, sunflower seeds and water



From Rodriguez-Saona et al.

Results IV. SPLAT Bioassays



Outstanding topics:

- 1) Investigating AK outside protected crops
- 2) Implementing attract-and-kill in other crops
- 3) Evaluating SPLAT with BMSB pheromone and in the field.



2.2.4 Repellents and mineral-derived compounds against BMSB



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 **Virginia Tech**

 **OSU Oregon State UNIVERSITY**

 **PENN STATE**

 **WASHINGTON STATE UNIVERSITY**

 **UNIVERSITY OF MARYLAND**

 **NC STATE UNIVERSITY**

Evaluation of kaolin and essential oils as natural repellents of BMSB

Tom Kuhar and Adam Morehead

Dept. of Entomology

Virginia Tech



Kaolin (Surround WP™)

- Kaolin [$\text{Al}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$] is a white, non-porous, non-swelling, low-abrasive, fine-grained, aluminosilicate mineral or clay that is derived from weathered feldspar & quartz
- a fine chemically inert powder that can safely be applied to plants to reduce heat stress, water loss, and sunscalding.



Surround® WP

Crop Protectant

Surround WP crop protectant forms a barrier film, which acts as a broad spectrum agricultural crop protectant for controlling damage from various insect and disease pests, a growth enhancer, and as a protectant against sunburn and heat stress.

ACTIVE INGREDIENT:

Kaolin 95.0%

OTHER INGREDIENTS: 5.0%

TOTAL: 100.0%

Essential oils

- Essential oils are secondary metabolite compounds produced by certain plants like rosemary & mint to deter feeding by insect pests.
- Zhang et al. (2014) recently showed that several essential oils including rosemary and spearmint oil had repellent activity against BMSB in the lab.
- Ecotec (EcosSMART Technologies, Inc.) is a commercially-available blend of essential oils

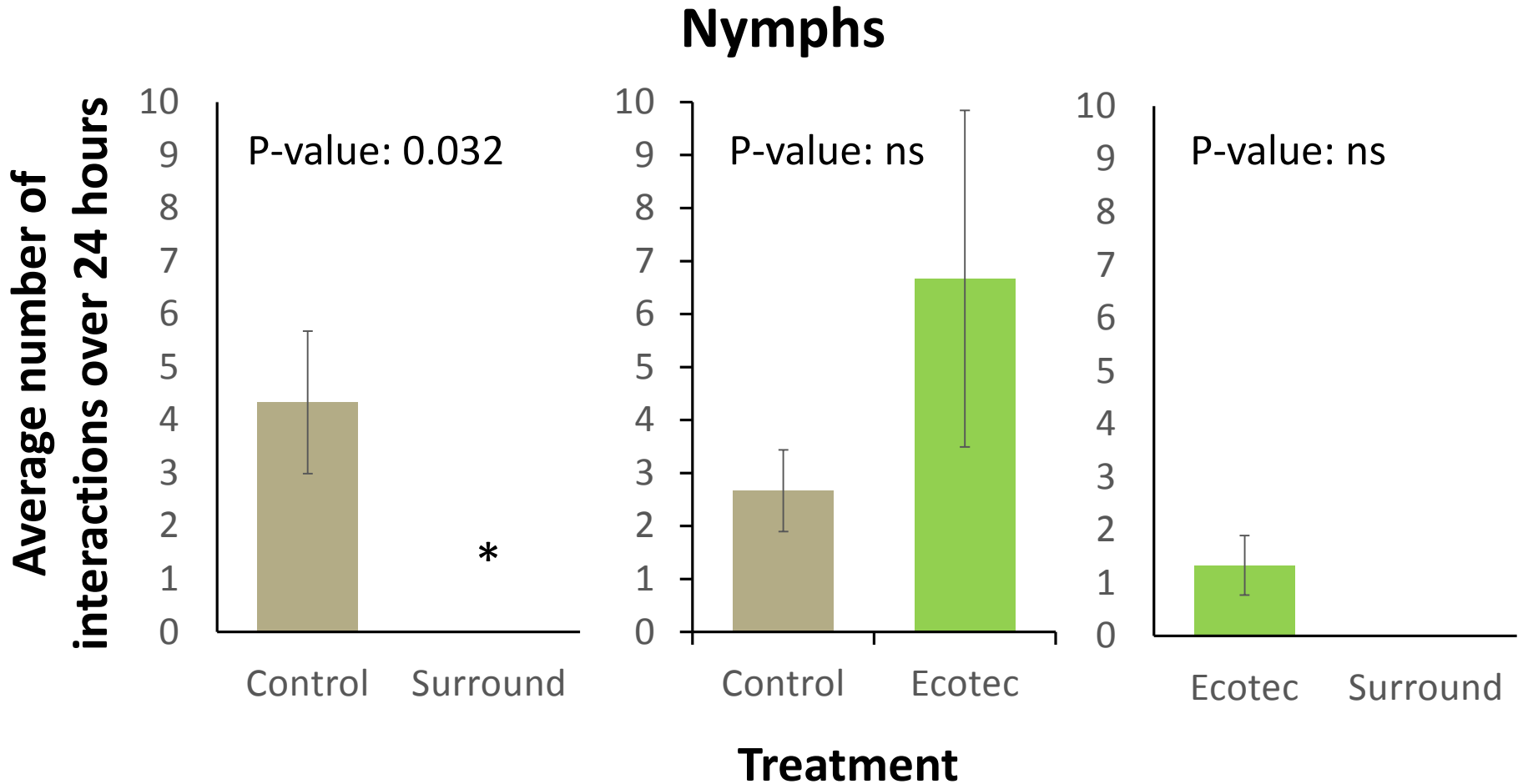


Repellent Choice Test Bioassays

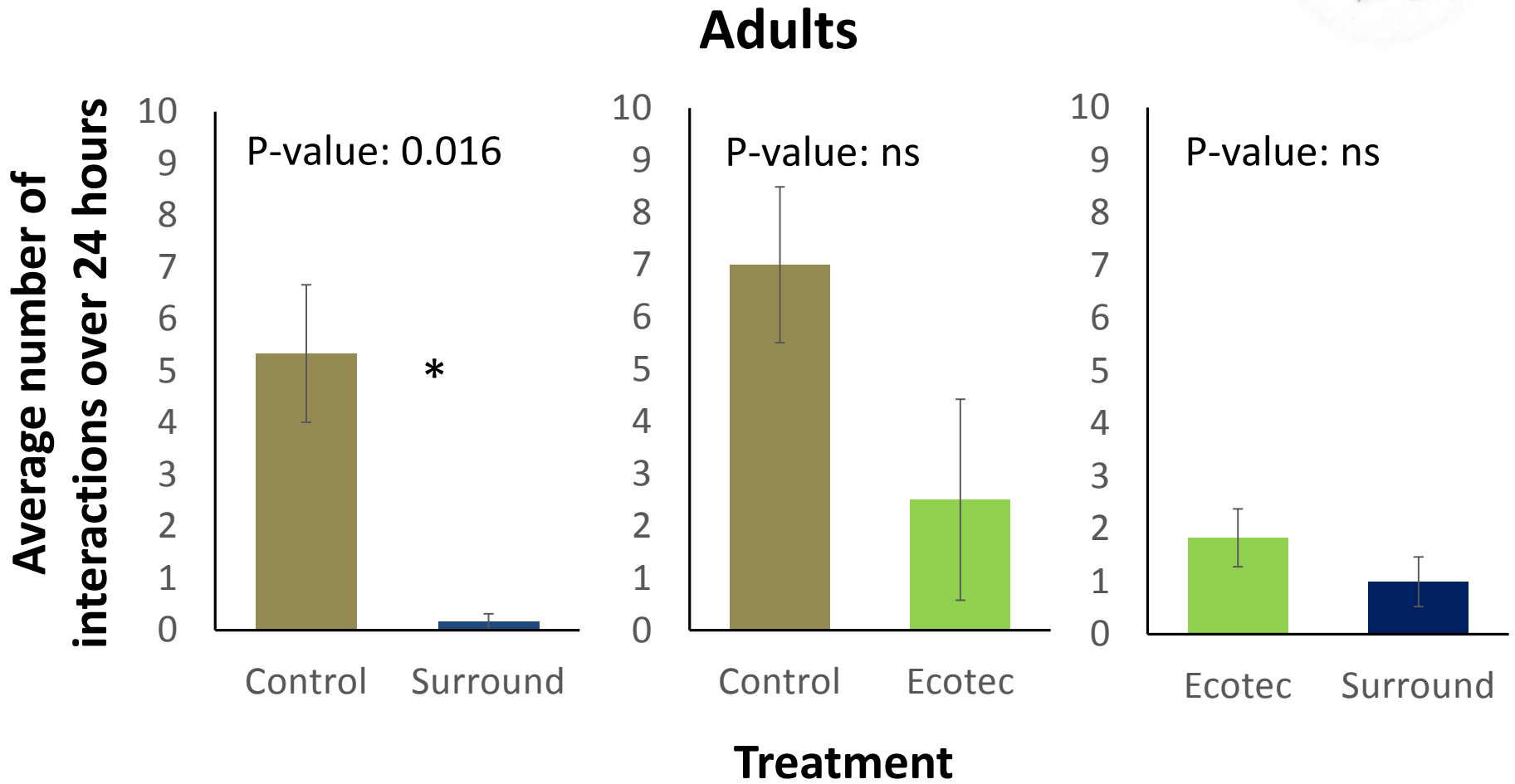
- Arena: 12” mesh rearing cube with two 90 mm Petri dishes on opposite corners
- *Paulownia* spp. leaves trimmed to fit dishes and treated with Surround WP, Ecotec, or Untreated
- Tomatoes placed in the center of trimmed leaves and given the same treatment as the leaf
- 10 insects were introduced into the arena (Adults or Nymphs)
- Checked at 20 min, 40 min, 1 hour, 2 hours, 4 hours, 6 hours, 8 hours, and 24 hours
- Analyzed with a paired T-test



Choice Test Bioassay Results



Choice Test Bioassay Results



Repellent Field Efficacy Trial

- Aristotle bell peppers planted into blocks and arranged into a randomized complete block design
- Plots were 4 rows x 20 feet
- Treatments were applied weekly with a 3 nozzle drop down boom attached to a CO₂ sprayer for the duration of each experiment
- Subsample per plot was 50 fruit in 2014 and 40 fruit in 2015
- Analyzed with ANOVA and Students T-test



Results Field Efficacy Tests

Treatment	Rate/acre	Cumulative average % damage caused by BMSB				
		8/29/2014	9/22/2014	8/12/2015	8/21/2015	8/28/2015
UTC		21.5% ± 3.6 A	13% ± 1.9 A	31.3% ± 3.9 A	33.8% ± 3.5 A	42.5% ± 3.2 A
Ecotec	64 fl oz	20.5% ± 4.9 A	4.5% ± 1.0 B	15% ± 3.6 AB	35.6% ± 4.6 A	35.6% ± 2.4 A
Surround	800 oz	3% ± 1.4 B	1% ± 1.4 C	10% ± 2.0 B	15.6% ± 2.9 B	8.8% ± 1.1 B
P value		0.015	0.003	0.024	0.027	0.001





Feeding Deterrent/Repellent for the Brown Marmorated Stink Bug

**Aijun Zhang, Yan Feng, Mark
Feldlaufer**

*Invasive Insect Biocontrol and
Behavior Laboratory*

*USDA - Agricultural Research
Service*

BARC-West, Beltsville, Maryland

Manguang Liu

*Hebei Forestry Academy of Science
Shijiazhuang, Hebei, China*

Kevin Ulrich

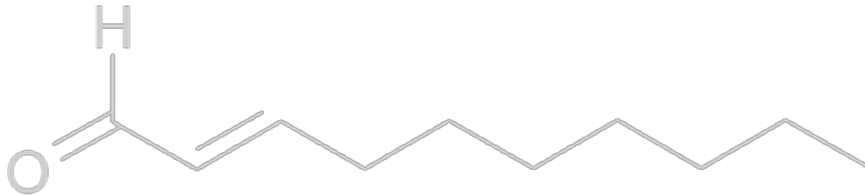
*Entomology Department
University of Maryland
College Park*

BMSB Feeding Deterrent/Repellent

BMSB Major Secretion Compounds

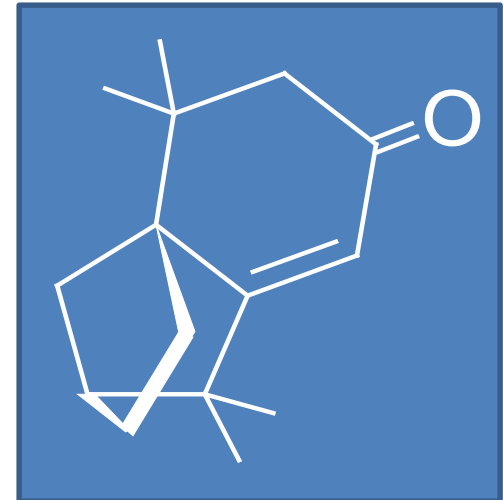


Tridecane (C₁₃)



E-2-Decenal (E2)

Mosquito & Tick Repellent

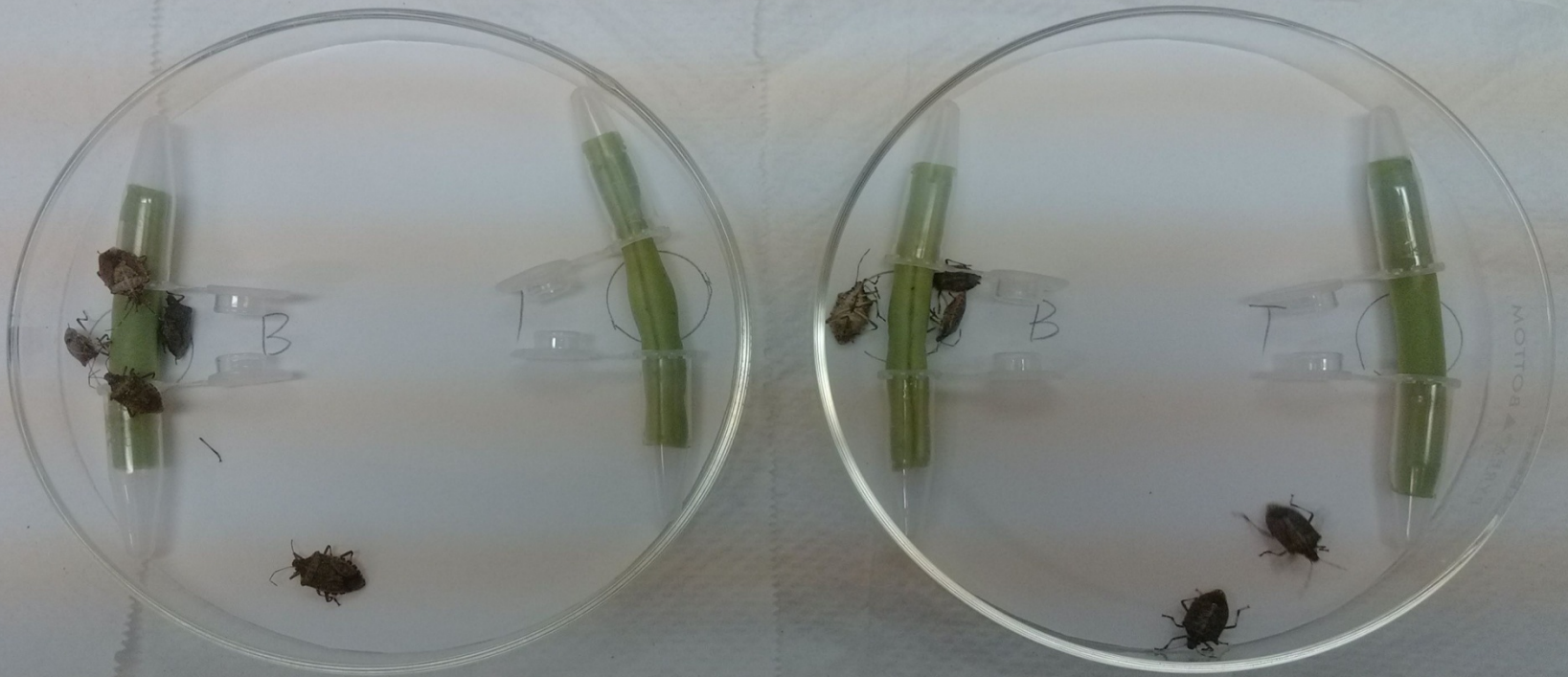


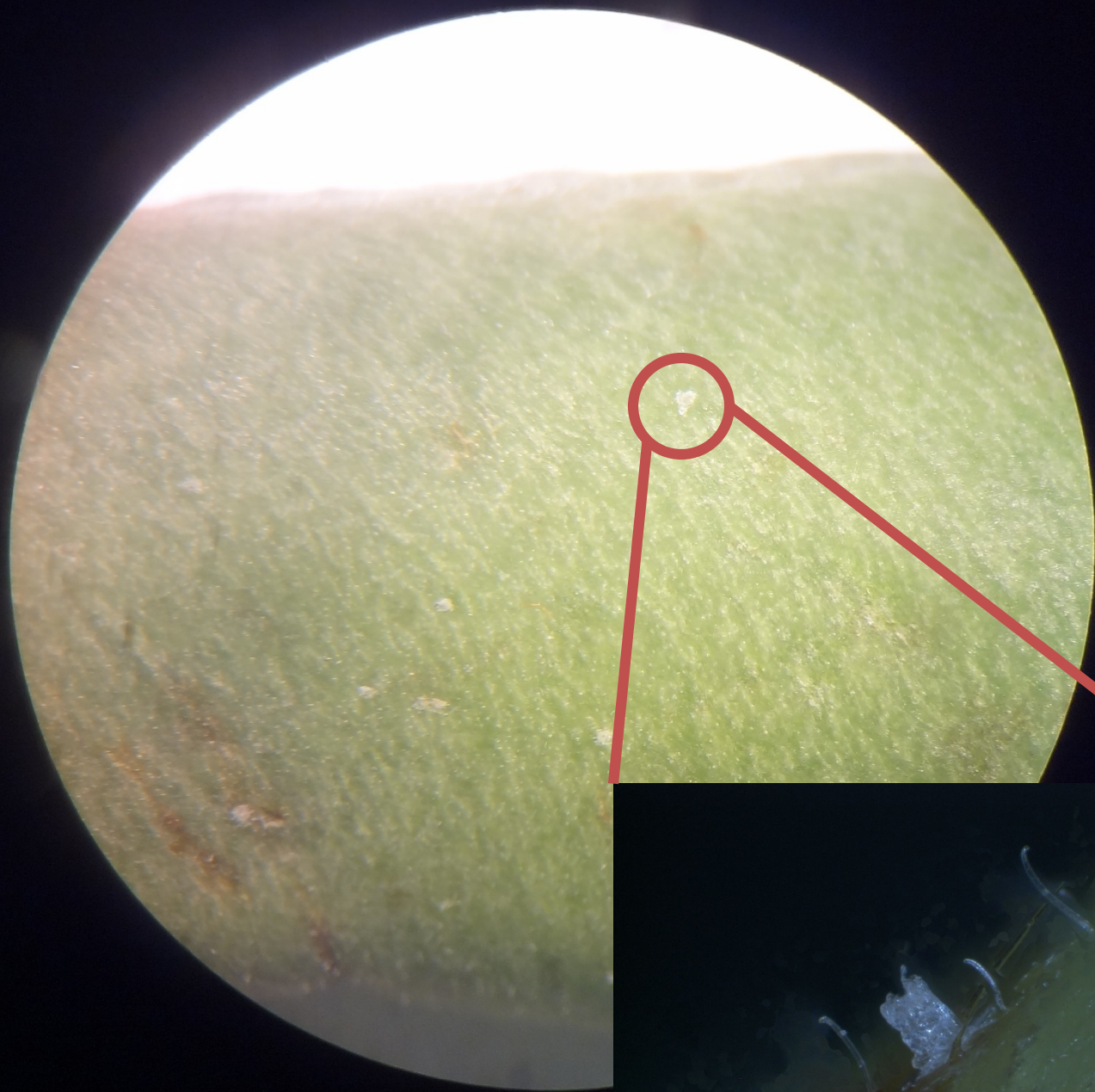
Isolongifolenone (En)

No Choice Test



Two Choice Test







Conclusions

- The major BMSB secretion compound (C13) has no effect on feeding activity.
- E2-decanal (E2) demonstrates dual functions in Petri Dish assay. At low concentration ($>100 \mu\text{g}$), it acts as feeding stimulant; while at high concentration ($<100 \mu\text{g}$), it acts as feeding deterrent.
- A blend of tridecane (C13) and E2-decanal (E2) (1:1 ratio) showed significant feeding deterrent activity.
- Isolongifolenone (En), a mosquito and tick repellent, exhibits strong feeding deterrent activity.
- A blend of C13, E2, and En (1:1:1 ratio) also exhibits strong feeding deterrent activity.
- Although identified feeding deterrents are natural products, they can not be directly applied on the plants (surfaces burning). New formulation is ready to be tested in the field in this fall.
- The identified feeding deterrents/repellents can be easily commercialized and used for protecting agricultural crops from *H. halys* damage in support of ongoing *H. halys* management programs.

Outstanding topics:

- 1) Further repellency studies to refine use in the field
- 2) Integrate with other management tactics



2.2.7 Cultural Techniques for IPM



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Interplanting of bell peppers with red clover



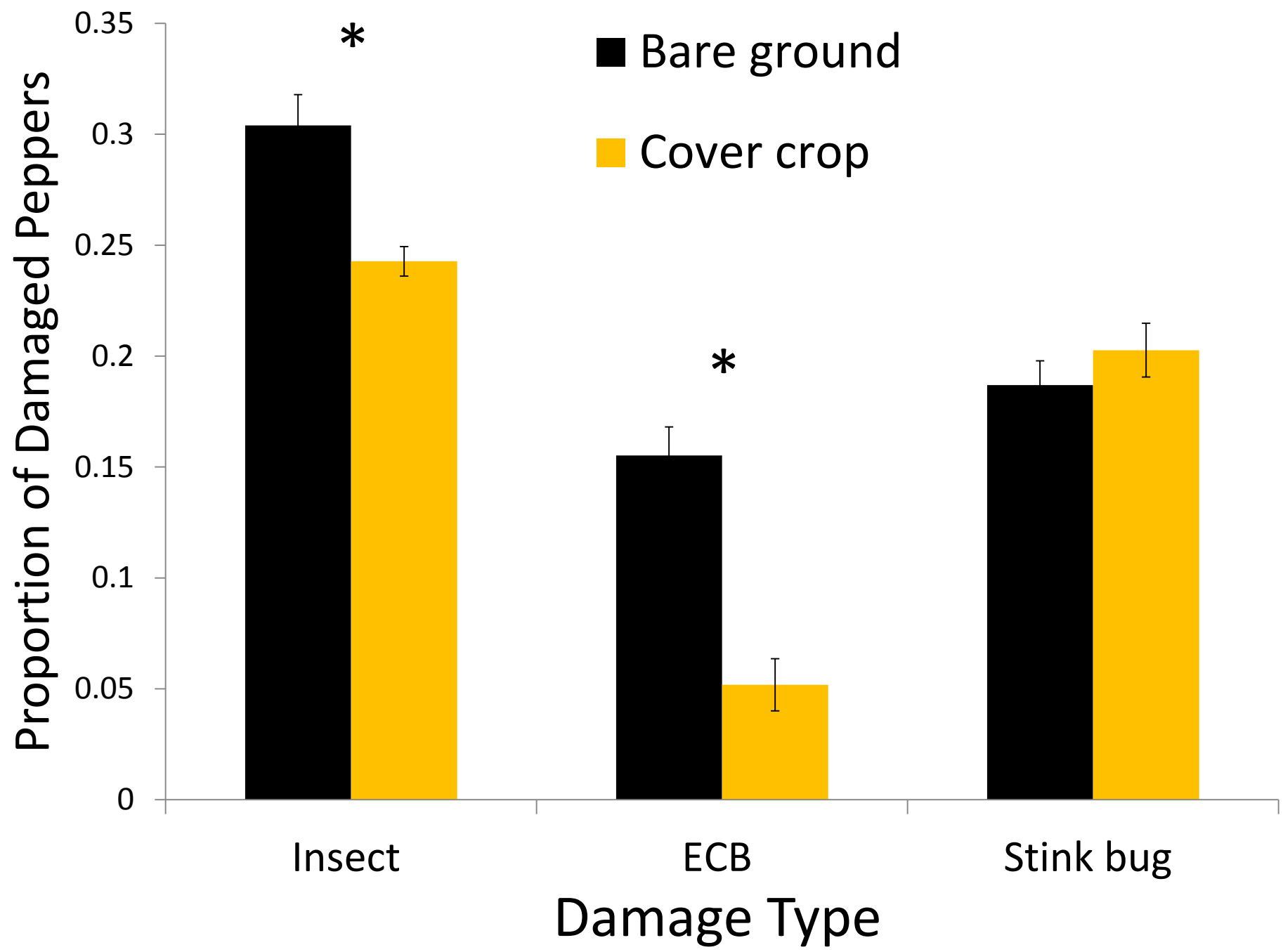
Hanna Kahl & Cerruti RR Hooks

University of MD, Dept. of Entomology

Objective

Determine the impact of inter-planting bell peppers with red clover on:

- 1) Stink bug egg mortality
- 2) Percentage of stink bug damaged fruits
- 3) Fruit yield



Cages to Exclude Stink Bugs



Two treatments: **caged vs. uncaged**

Conspere stink bug used as surrogate

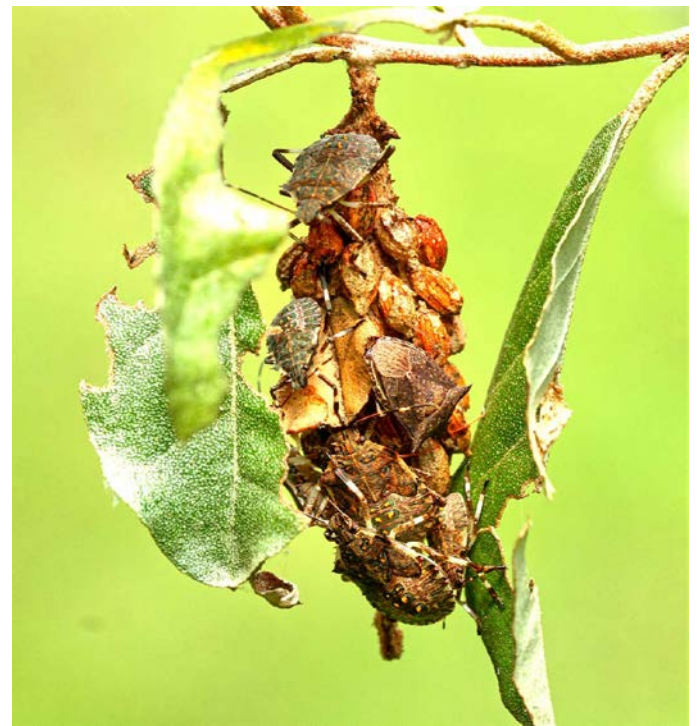
1,932 fruit harvested: no damage in either treatment

E. Beers et al.



Outstanding topics:

- 1) Verify cultural control results with additional cover crops and compare with trap cropping
- 2) Repeat exclusion experiments under higher population pressure



2.2.8 RNAi against BMSB



©WBI Herzhunger



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Amylase in BMSB saliva

- In cooperation with DOW Chemical, proteome data has been re-analyzed with the newly available BMSB genome(www.hgsc.bcm.edu/brown-marmorated-stink-bug-genome-project)



- We are focusing on 2 amylases:

1. HHAL004834 is an α -amylase identified in both watery saliva and the salivary sheath
2. HHAL001011 is an α -amylase identified in watery saliva only



Salivary sheaths on green bean (left) and tomato (right)

- Currently we are using the SMARTer RACE technique to clone the full length genes and obtain complete sequence information
- The sequence information will be used to create small silencing RNA to suppress amylase in the saliva

Outstanding topics:

- 1) Create effective RNAi materials for use against BMSB in the field



Acknowledgements



United States Department of Agriculture
National Institute of Food and Agriculture

- USDA-ARS, USDA NIFA SCRI # 2011-51181-30937,
- USDA-APHIS



BMSB SCRI CAP Team

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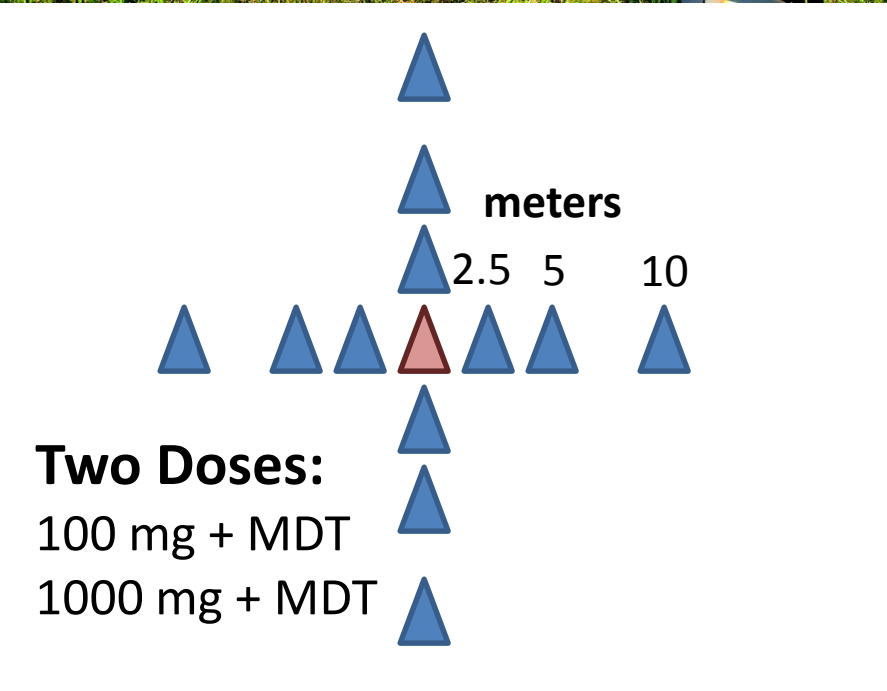
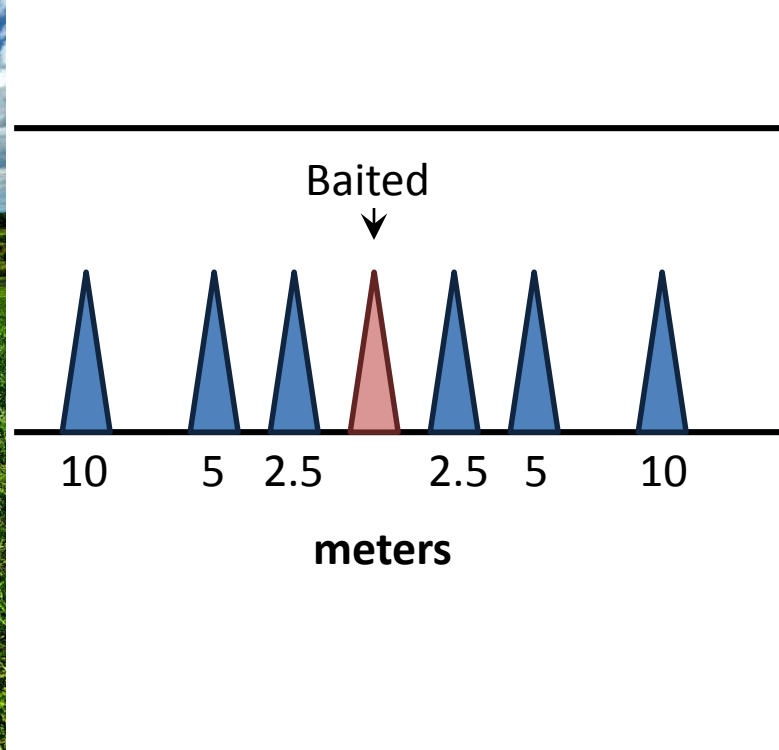
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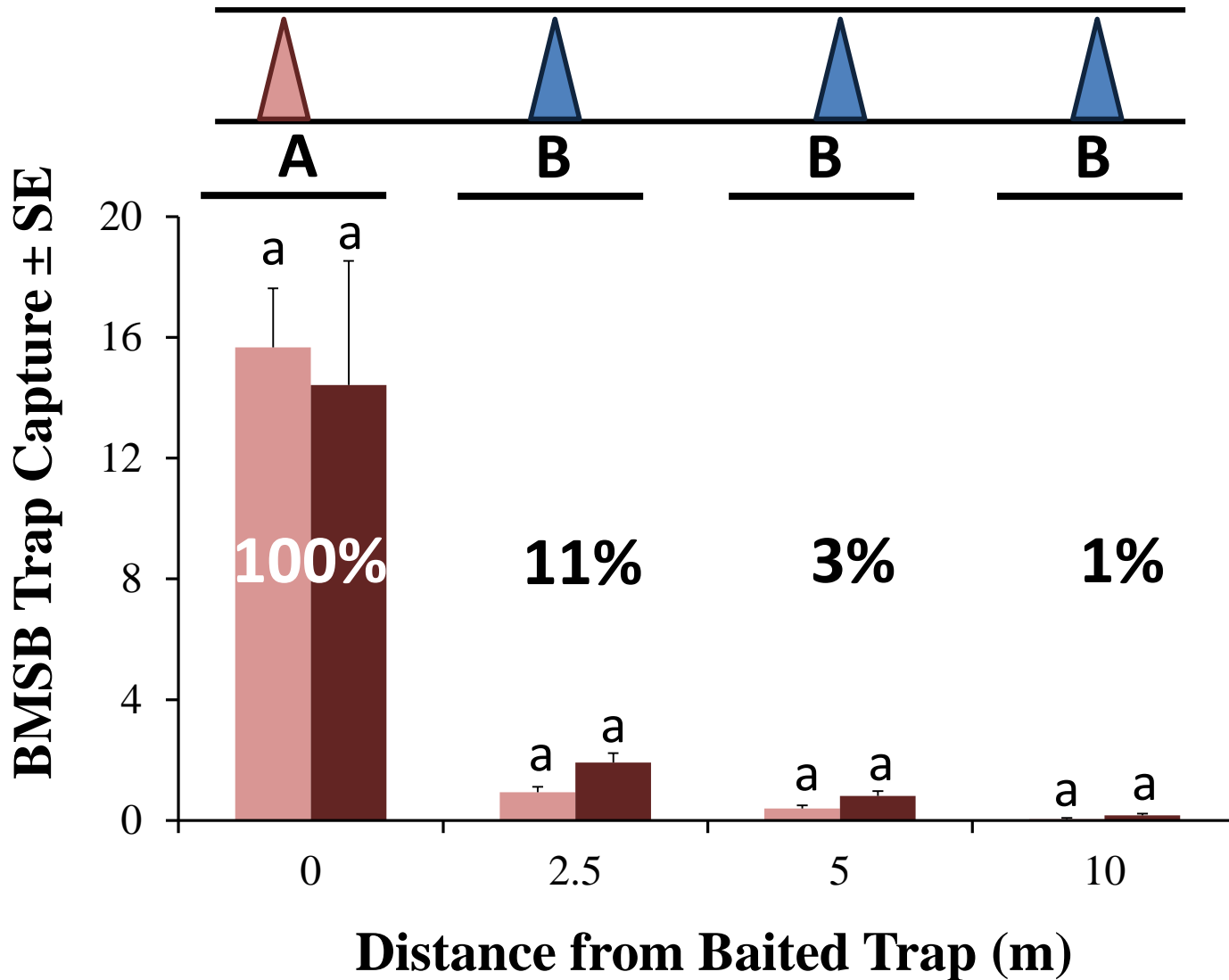
Thank you for your attention!



In the field one morning...



Adults



- 100 mg
- 1000 mg

ANOVA

Dose

$$F_{1,382} = 1.44$$

$$P < 0.23$$

Distance

$$F_{3,382} = 17.1$$

$$P < 0.0001$$

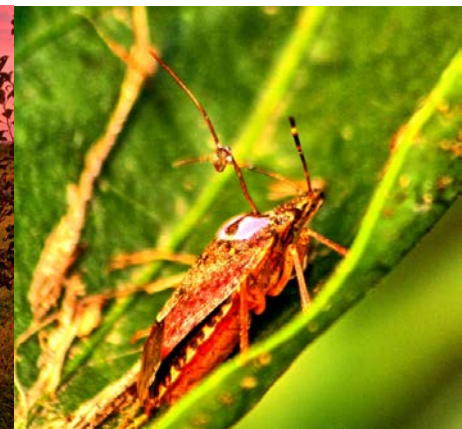


Apple Tree



Mowed Grass

Measuring: Retention time, distance from the release point





Apple Tree



Mowed Grass

Unbaited



Apple Tree



Mowed Grass

Baited

Measuring: Retention time, distance from the release point





Apple Tree



Mowed Grass



Apple Tree



Mowed Grass

Unbaited

Baited

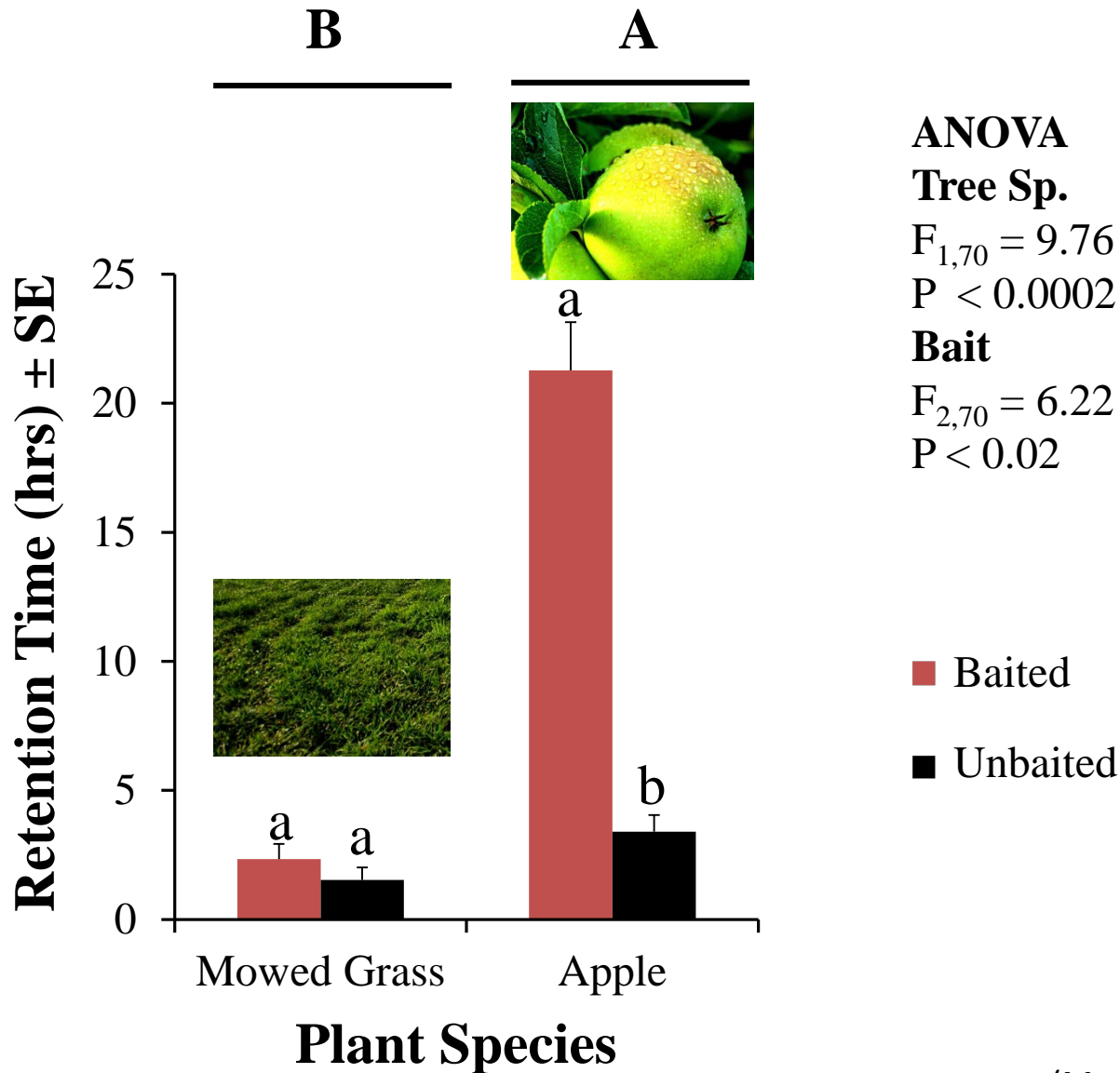
Sampling after release

- ↓
- 1 hr
- ↓
- 3 hrs
- ↓
- 6 hrs
- ↓
- 24 hrs

Measuring: Retention time, distance from the release point



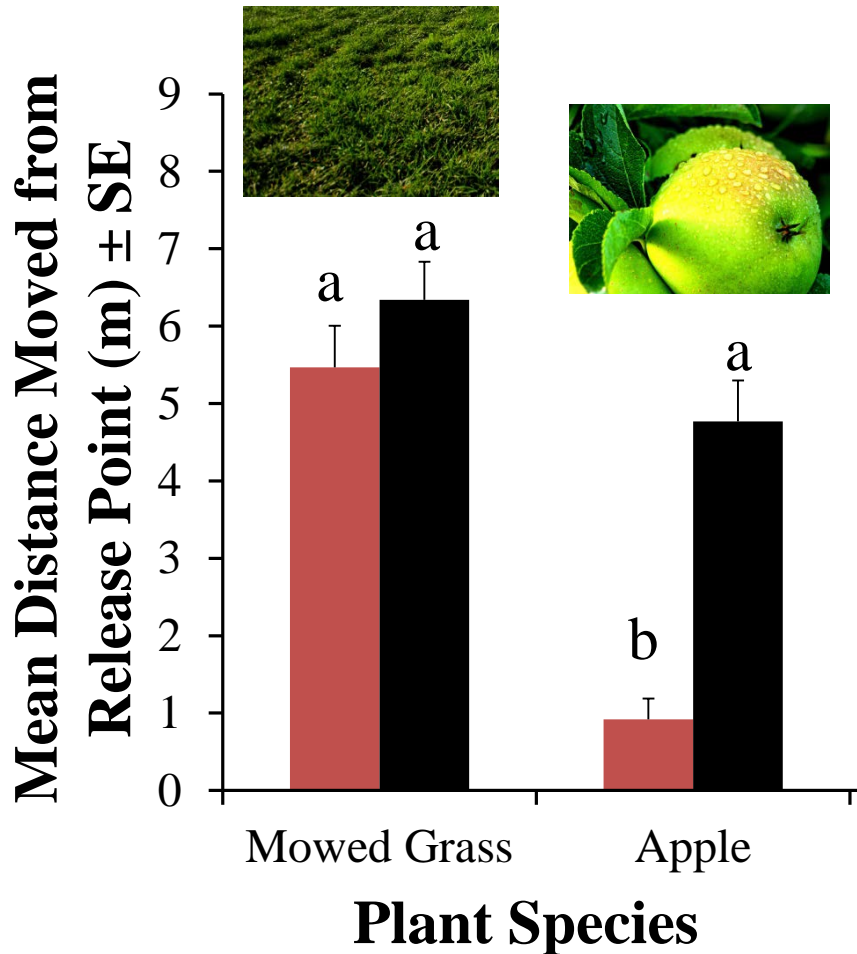
Retention Time



Distance Moved

A

B



ANOVA

Tree Sp.

$F_{2,306} = 83.7$

$P < 0.0001$

Bait

$F_{1,306} = 84.1$

$P < 0.0001$

■ Baited

■ Unbaited