Objective 1: Nutritional ecology including diet optimization, salivary gland characterization, gut symbionts and colony procedures

Funding

USDA

United States Department of Agriculture

National Institute of Food and Agriculture

Specialty Crop Research Initiative
Grant #2011-01413-30937

Collaborating Institutions

University of Delaware

ARS

Penn State

The State University of New Jersey Rutgers

Northeastern IPM Center

Washington State University

Cornell University

Oregon State University

University of Maryland

Virginia Tech

NC State University

StopBMSB.org

Summary of Work to Date

- Nutritional profile dynamics
  - Nik Wiman and Victoria Skillman (Oregon State), Jana Lee (USDA)
- Diet suitability
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- Salivary gland characterization
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Nutrient profiles of BMSB

- Understanding the nutrient profile of BMSB in the wild can potentially pinpoint vulnerable periods, and predict how plant resources are utilized.
- No information on nutrient profiles of naturally-occurring adult BMSB in North America.
- Objective: Nutrient dynamics of wild BMSB
    1. As they emerge from overwintering (March-June)
    2. Through the field season (May-Sept)

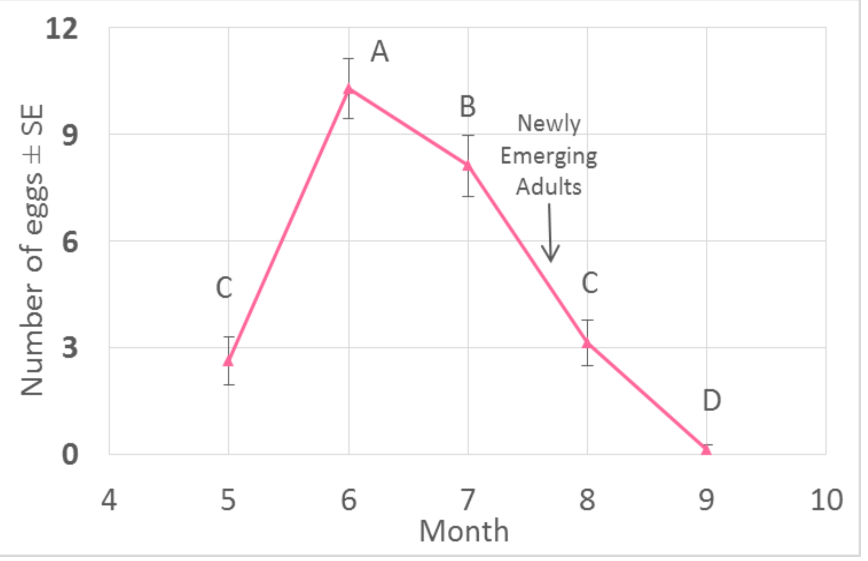
BMSB Collections

- Overwintering: Adults collected as they emerged from boxes Mar-June
- Season: Beat holly trees from 5 locations in Oregon May-Sept
- Measurements
  - Weight
  - Prothorax width
  - Ovary/spermathecal
  - Nutrients (lipid, glycogen, sugars)

Skillman, Wiman, Lee – USDA/OSU Oreg

Eggs

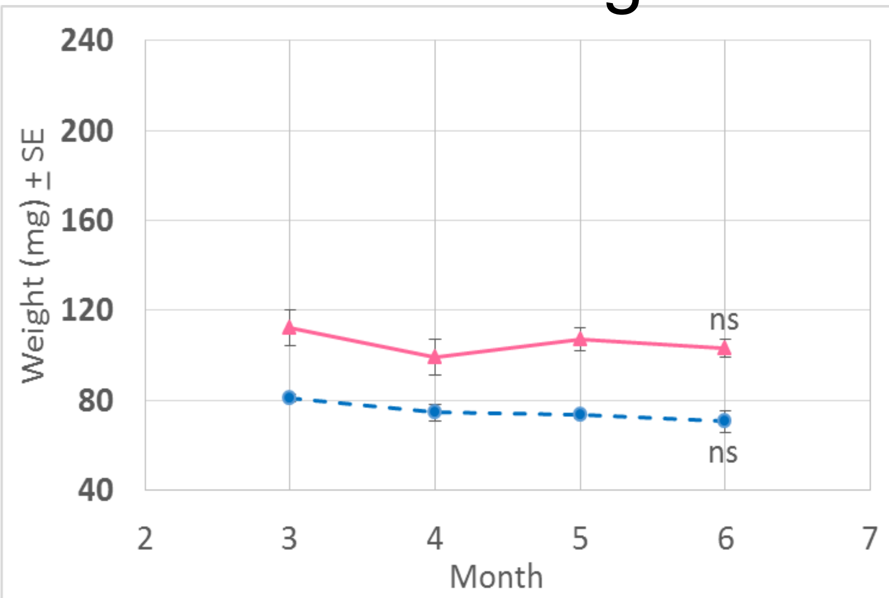
- Overwintering: No eggs found in adult ♀ as they emerged from boxes
- Season: Among ♀ from holly, peak egg load was observed in June



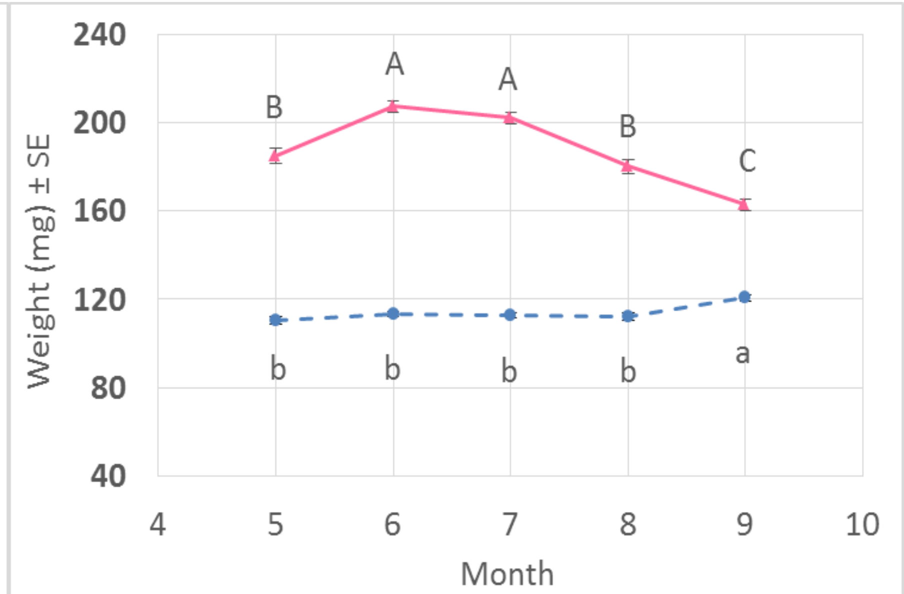
Skillman, Wiman, Lee – USDA/OSU Oregon

Weight

Overwintering-box



Season-holly

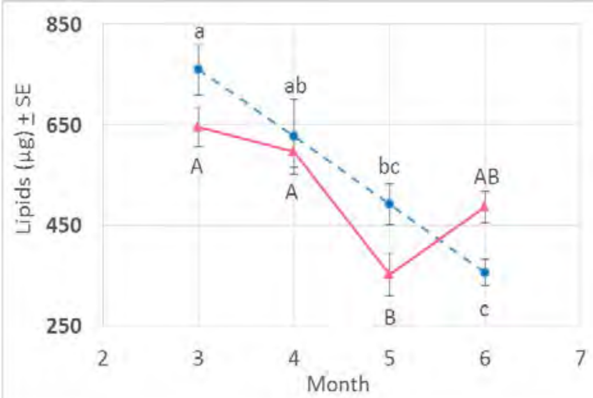


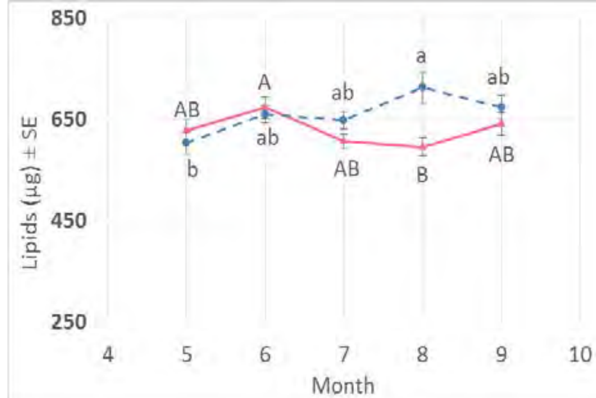
- For each sex and collection type (overwintering, holly), comparisons were made between months.
- ♀ (red) from holly weighed more in early summer
- ♂ (blue) weigh more in September

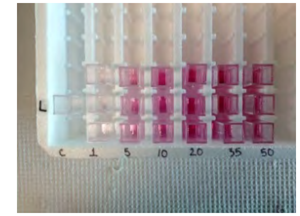
Overwintering-box

Season-holly

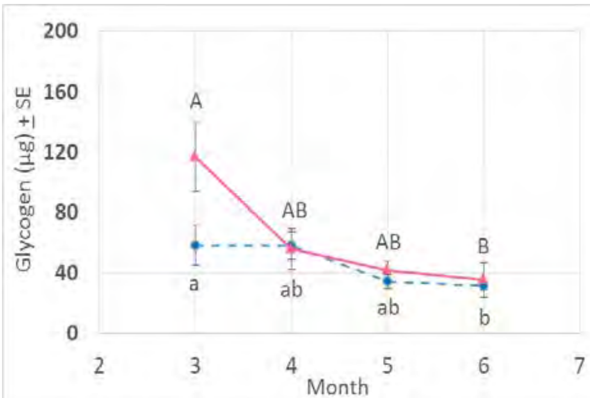
Lipids

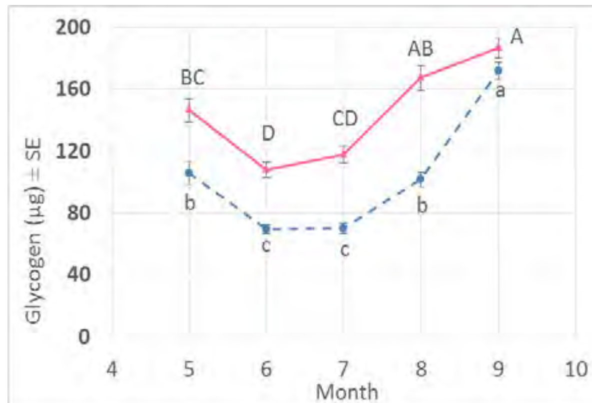


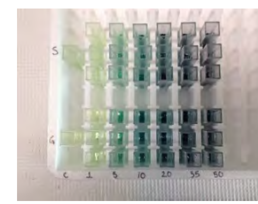




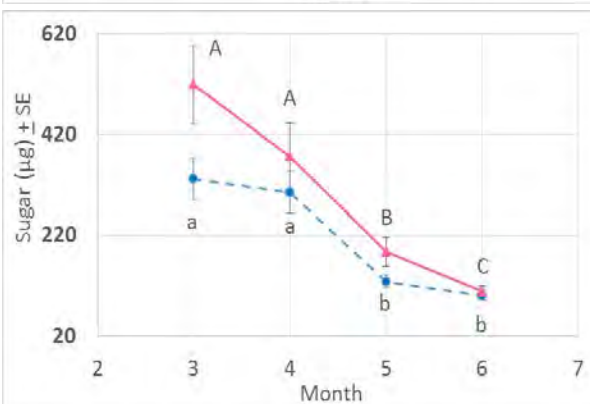
Glycogen

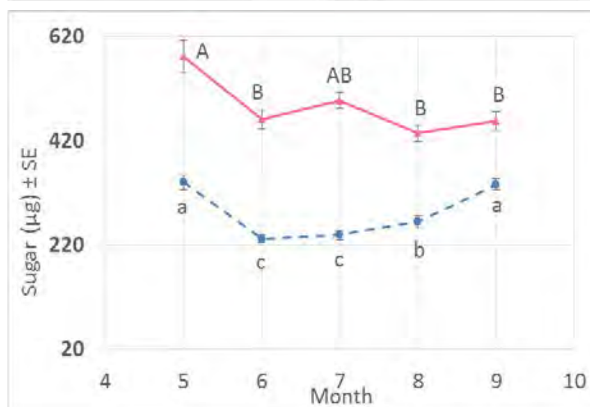






Sugars





Summary

- Adults emerging from overwintering exhibit a steady decline in lipids, glycogen, and sugars as they emerged later in the season. This suggests that overwintering for longer periods of time uses up more nutrient reserves.

- Adults that emerged from overwintering in May-June had numerically lower weights and nutrient reserves than their counterparts collected from holly at the same time. This suggests that feeding on host plants may have replenished their reserves.

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Determine the relative suitability of single and mixed diets of selected wild and fruit tree hosts on BMSB development and survivorship

Tree fruit hosts:

Wild hosts:

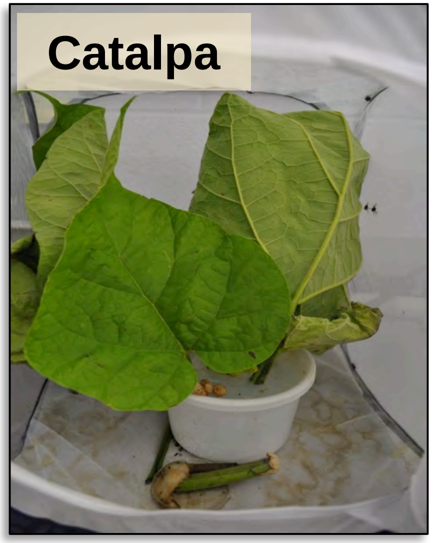
Apple

Peach

Catalpa

Tree of heaven

Single



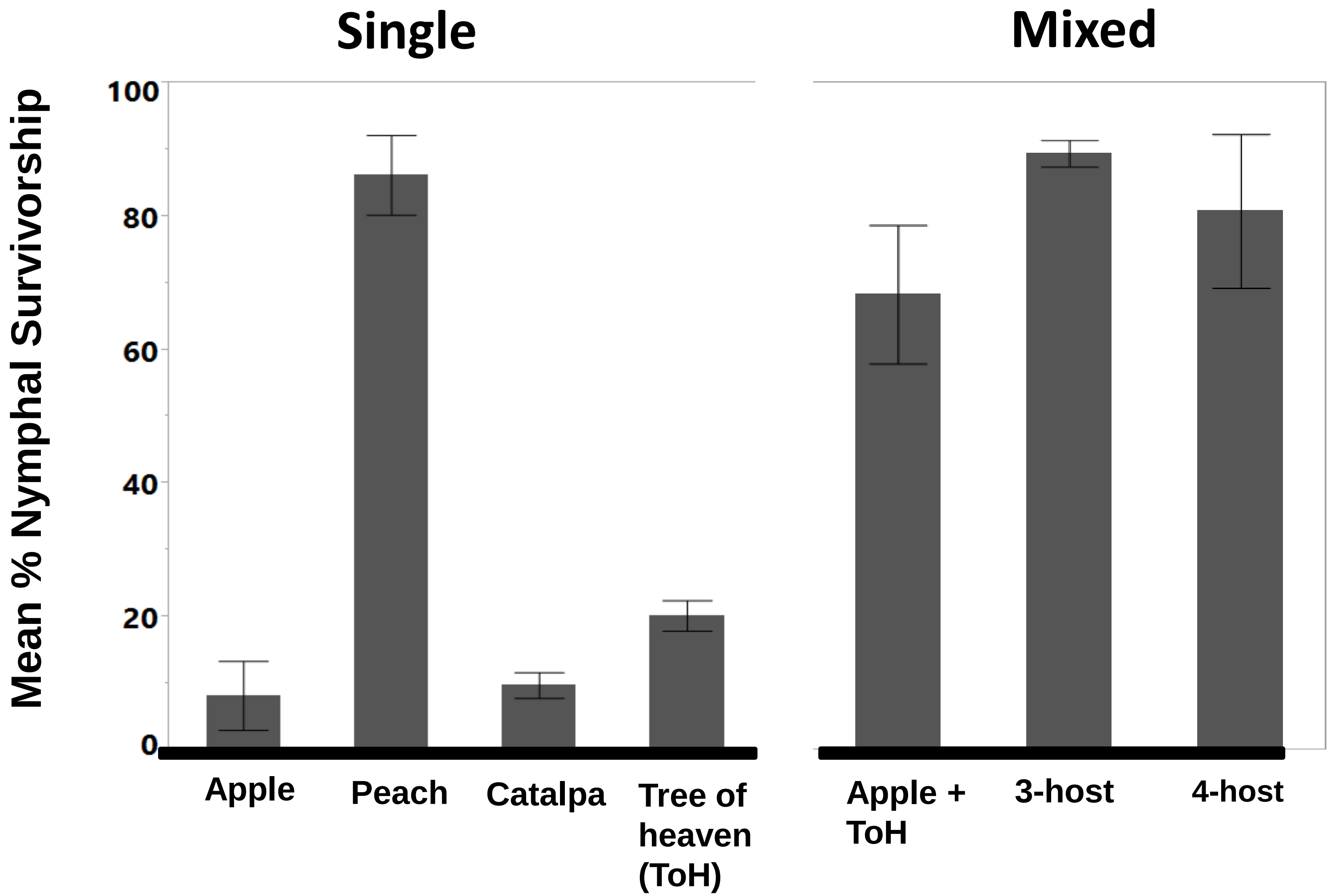
Mixed

Methods

- 1 egg mass/treatment (replicated 4x)
- Checked daily until adult eclosion
- Field-collected plant materials replaced regularly
- Measured:
  - Survivorship
  - Development time
  - Adult live body weight and size (pronotal width)
  - Sugar, lipid and protein contents of the adults

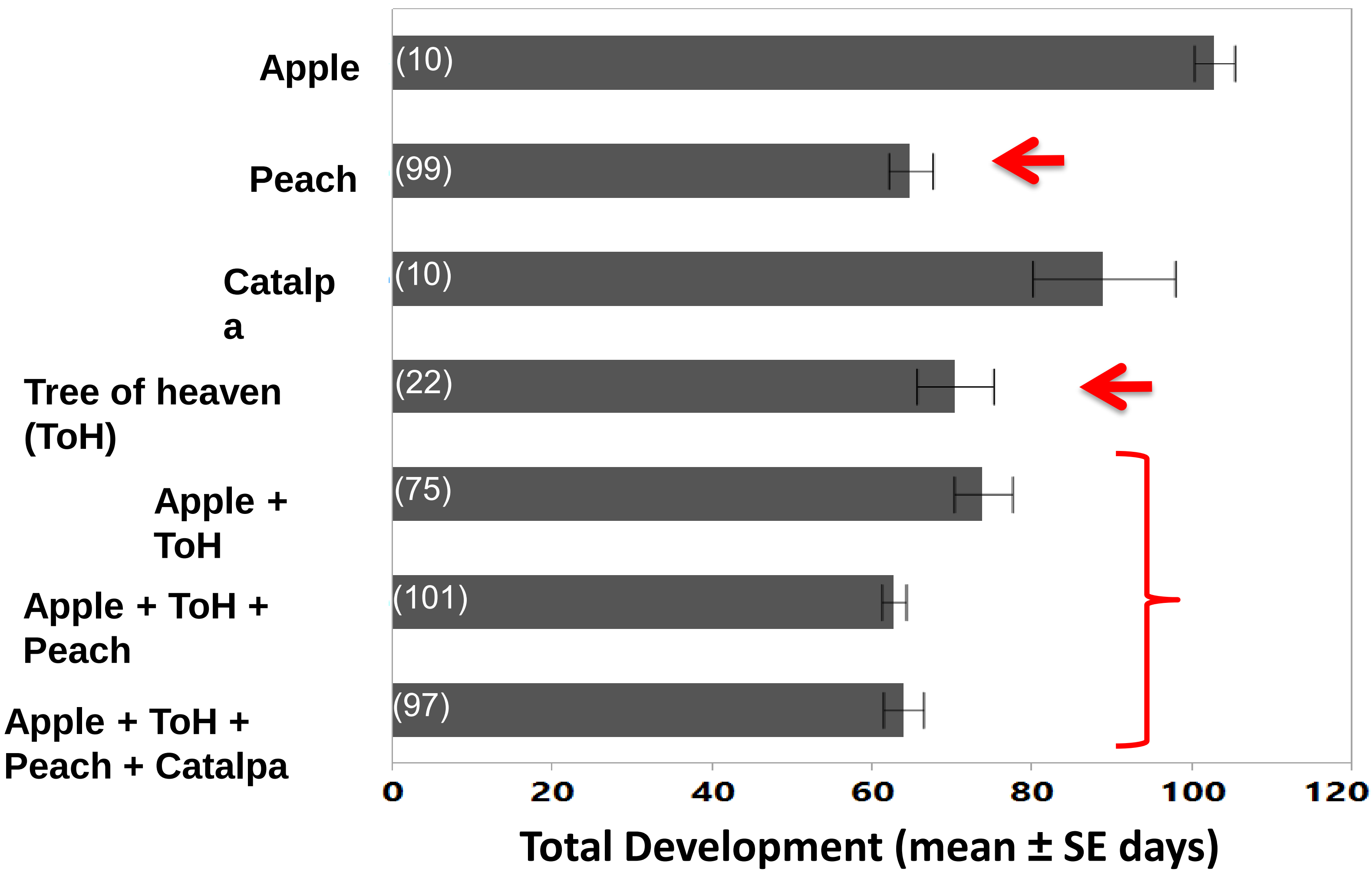
Results

- Mixed diets proved to be optimal for nymphal survivorship
- Peach is a highly suitable single host



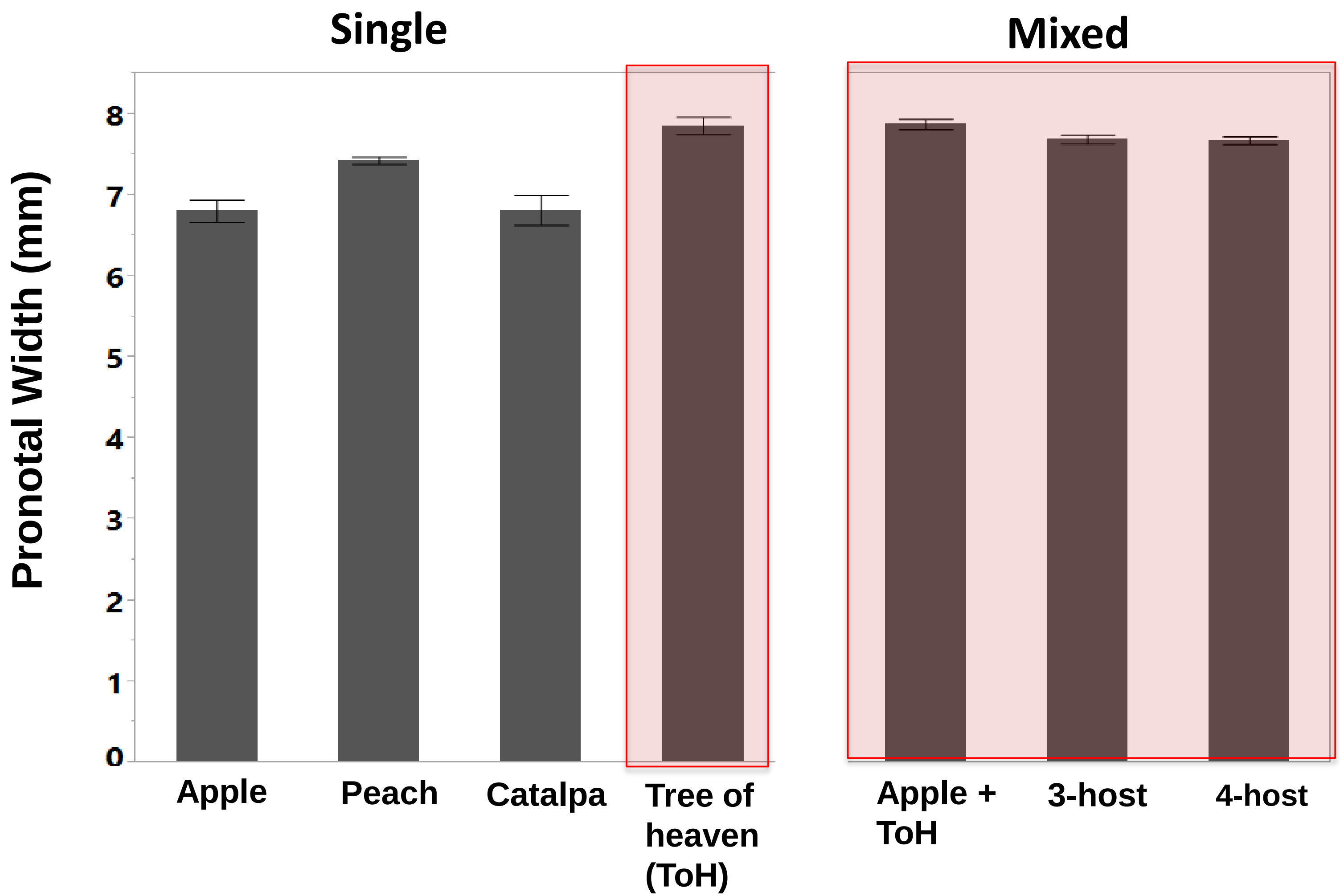
Results: Developmental Time

- BMSB developed faster on mixed diets and single diets of peach and ToH
- Longer development on single diets of apple and catalpa



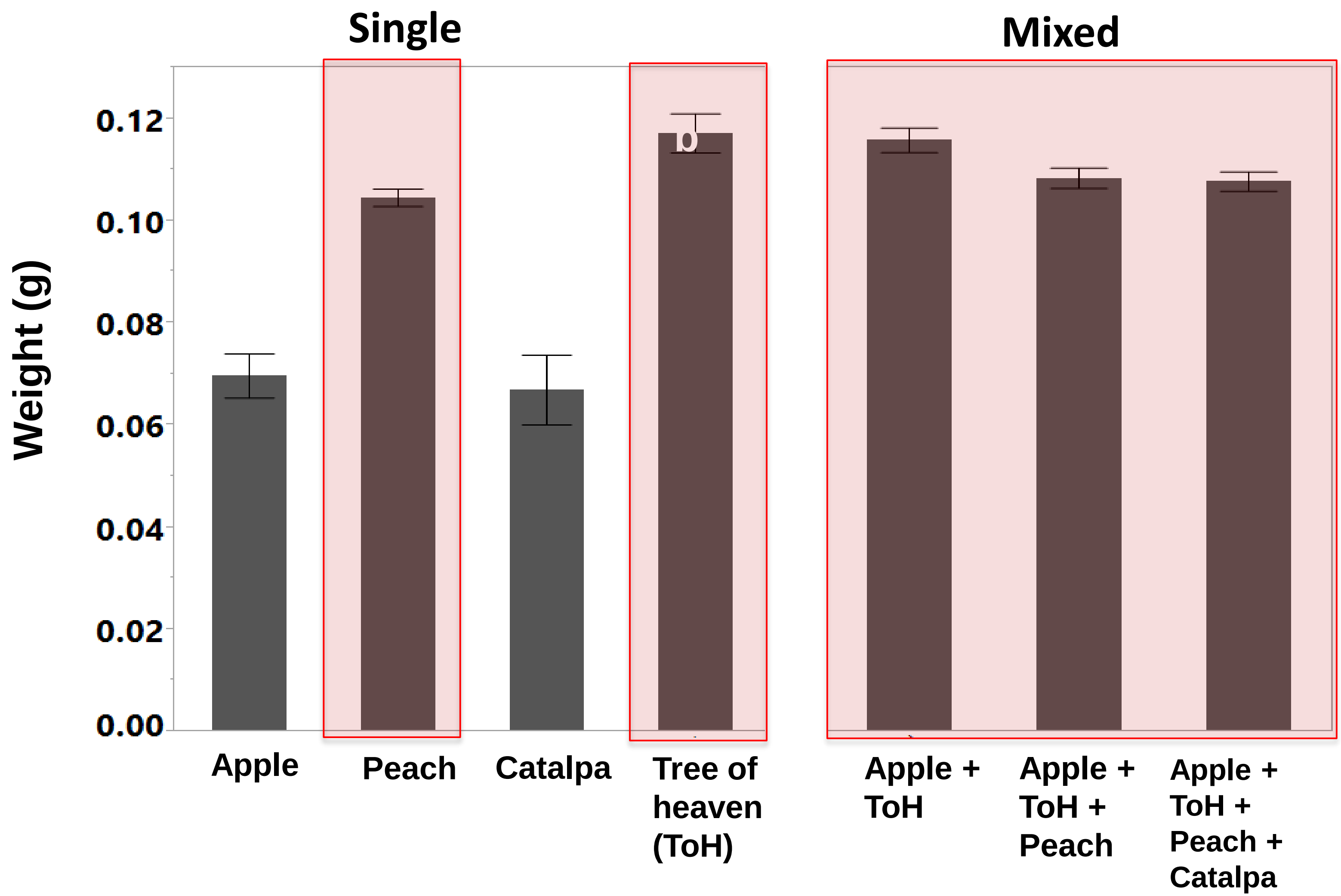
Results: Size

- Adults reared on mixed diets and ToH (single diet) were larger



Results: Weight

- Adults reared on mixed diets and single diets of ToH and Peach were heavier



Results: Nutrient contents of adults

- Nutrient levels of adults varied among different diets

		FEMALES		
Diet **treatments**	**N**	**Sugar (mg/mL)**	**Lipid (mg/mL)**	**Protein (mg/mL)**
Apple	2	0.07 ± 0.04*	0.71 ± 0.03*	4.32 ± 2.77*
Peach	15	0.19 ± 0.02a	0.85 ± 0.04bc	11.01 ± 0.79a
Catalpa	2	0.08 ± 0.03*	0.81 ± 0.25*	7.85 ± 4.76*
ToH	7	0.03 ± 0.01b	1.96 ± 0.54a	11.22 ± 0.61a
Apple + ToH	15	0.18 ± 0.03a	1.50 ± 0.22ab	11.47 ± 0.75a
3-host[a]	15	0.18 ± 0.02a	0.83 ± 0.06c	10.02 ± 0.69a
4-host[b]	15	0.19 ± 0.04a	0.89 ± 0.08bc	9.84 ± 0.71a
One-way ANOVA		$P = 0.031$	$P = 0.0002$	$P = 0.42$

		MALES		
Diet treatments	**N**	**Sugar (mg/mL)**	**Lipid (mg/mL)**	**Protein (mg/mL)**
Apple	7	0.07 ± 0.01bc	0.51 ± 0.03d	10.16 ± 1.82a
Peach	15	0.18 ± 0.02a	0.77 ± 0.07cd	10.24 ± 0.65a
Catalpa	8	0.03 ± 0.01c	0.60 ± 0.03cd	3.11 ± 0.40b
ToH	14	0.04 ± 0.01c	1.16 ± 0.12ab	9.11 ± 0.43a
Apple + ToH	15	0.12 ± 0.03ab	1.29 ± 0.14a	9.88 ± 0.53a
3-host[a]	15	0.19 ± 0.01a	1.00 ± 0.07abc	8.59 ± 0.59a
4-host[b]	15	0.19 ± 0.04a	0.89 ± 0.08bc	9.84 ± 0.71a
One-way ANOVA		$P < 0.0001$	$P < 0.0001$	$P < 0.0001$

Summary

- Mixed diets proved to be optimal for nymphal survivorship and development
- Nymphs reared on mixed diets and ToH developed faster and they resulted into bigger and heavier adults
- Peach appeared to be the most suitable single host for BMSB development among the host plants tested
- Nutrient levels of adults that developed from nymphs reared on different diets were different across treatments
- Results suggest that *H. halys* optimizes diet by utilizing multiple hosts during its development

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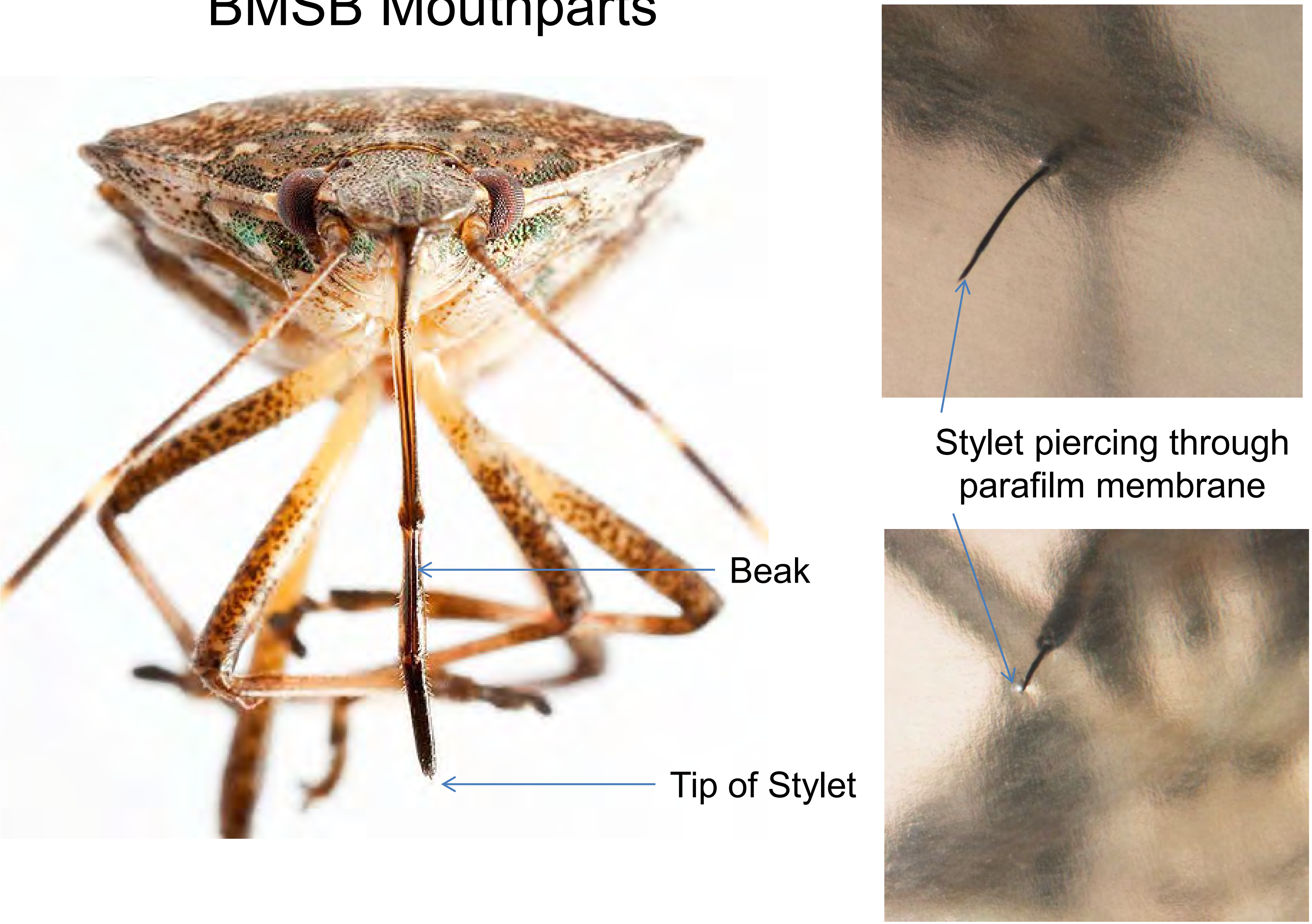
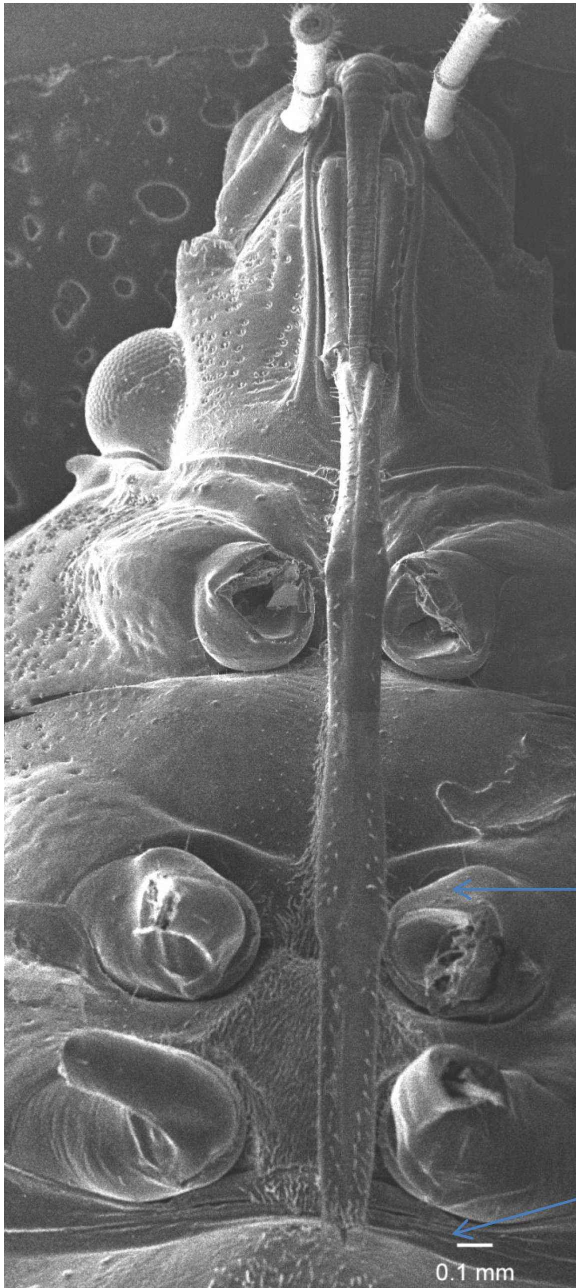
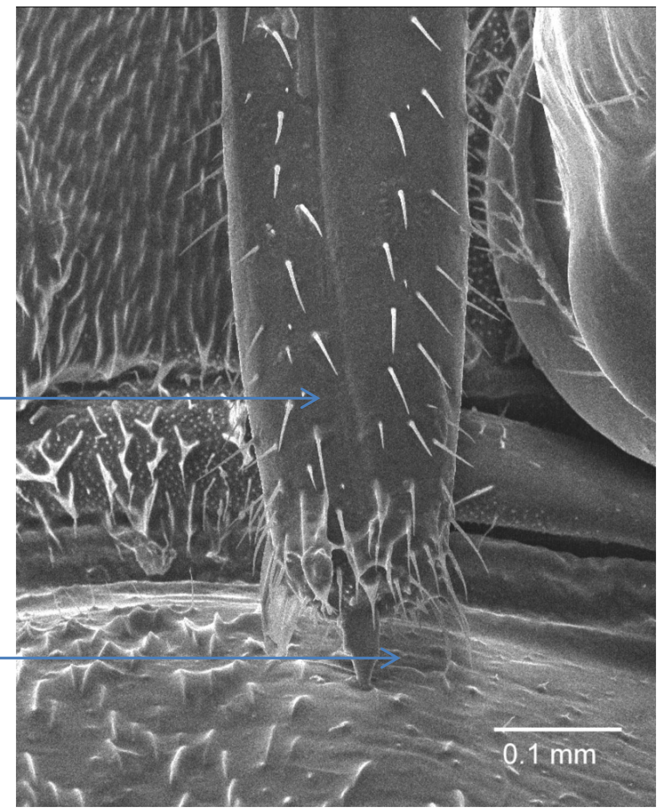
BMSB Mouthparts

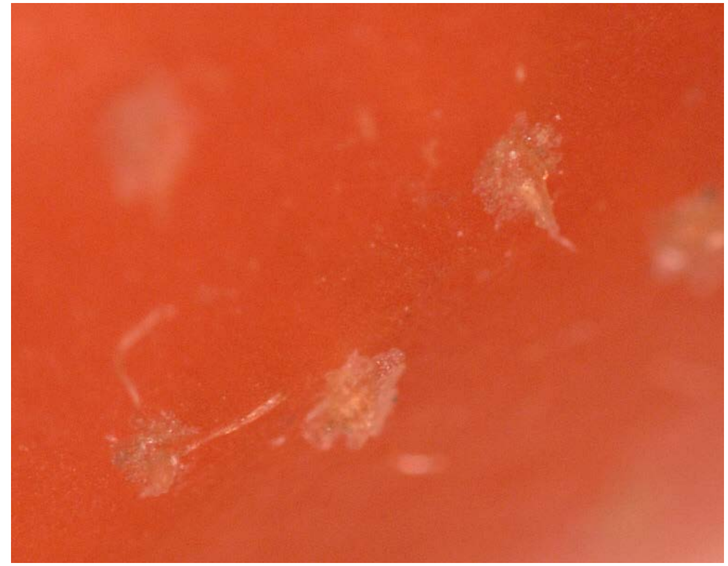


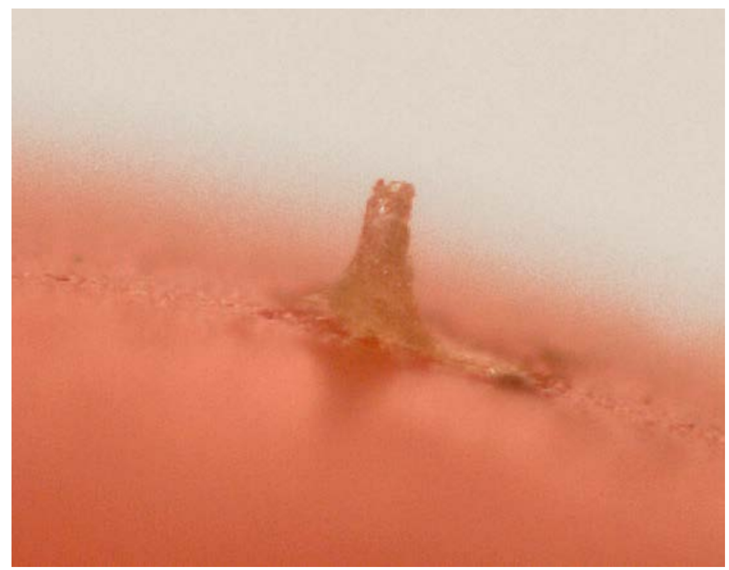
Scanning Electron Micrograph of BMSB mouthparts



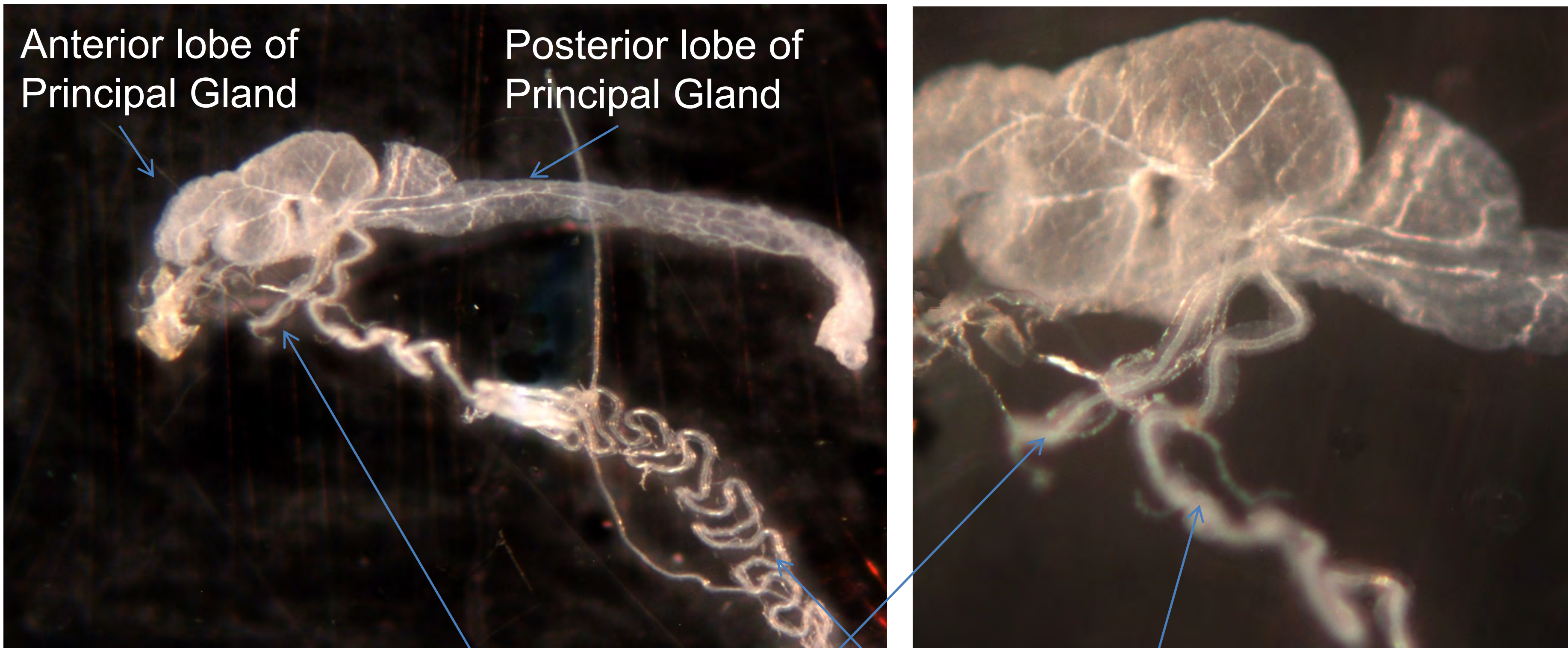


BMSB Salivary Sheaths



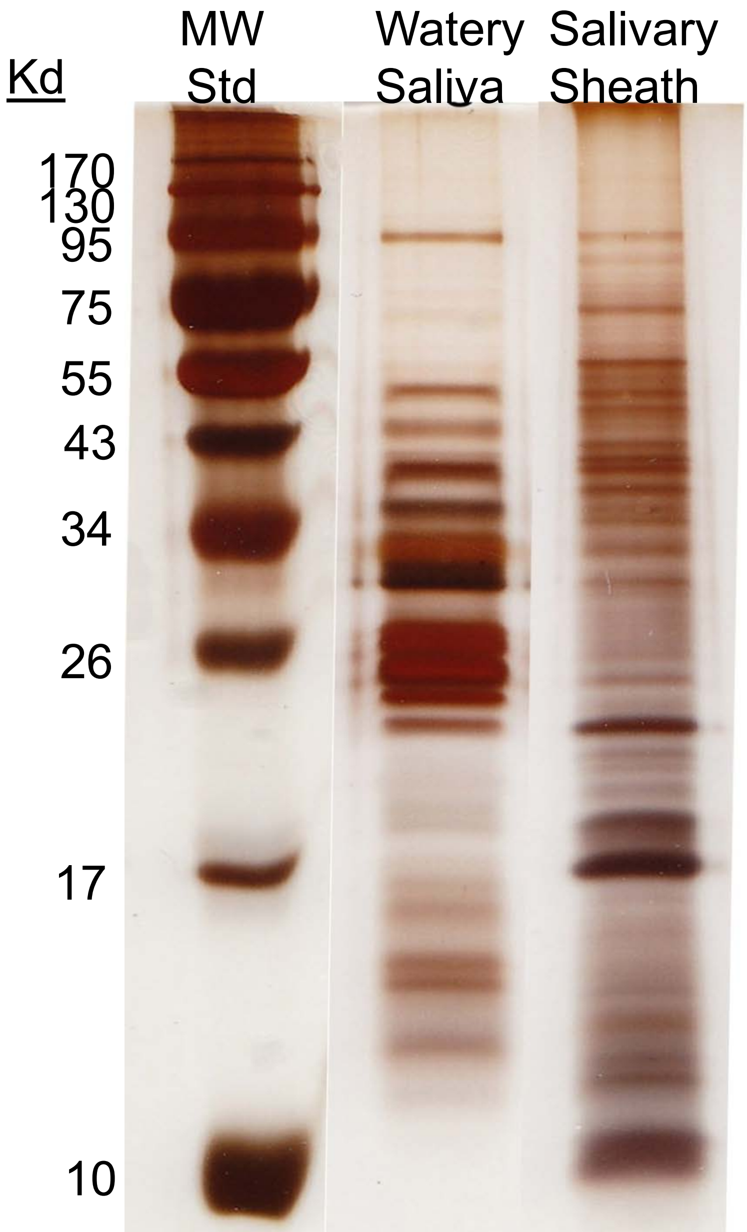


BMSB Salivary Glands



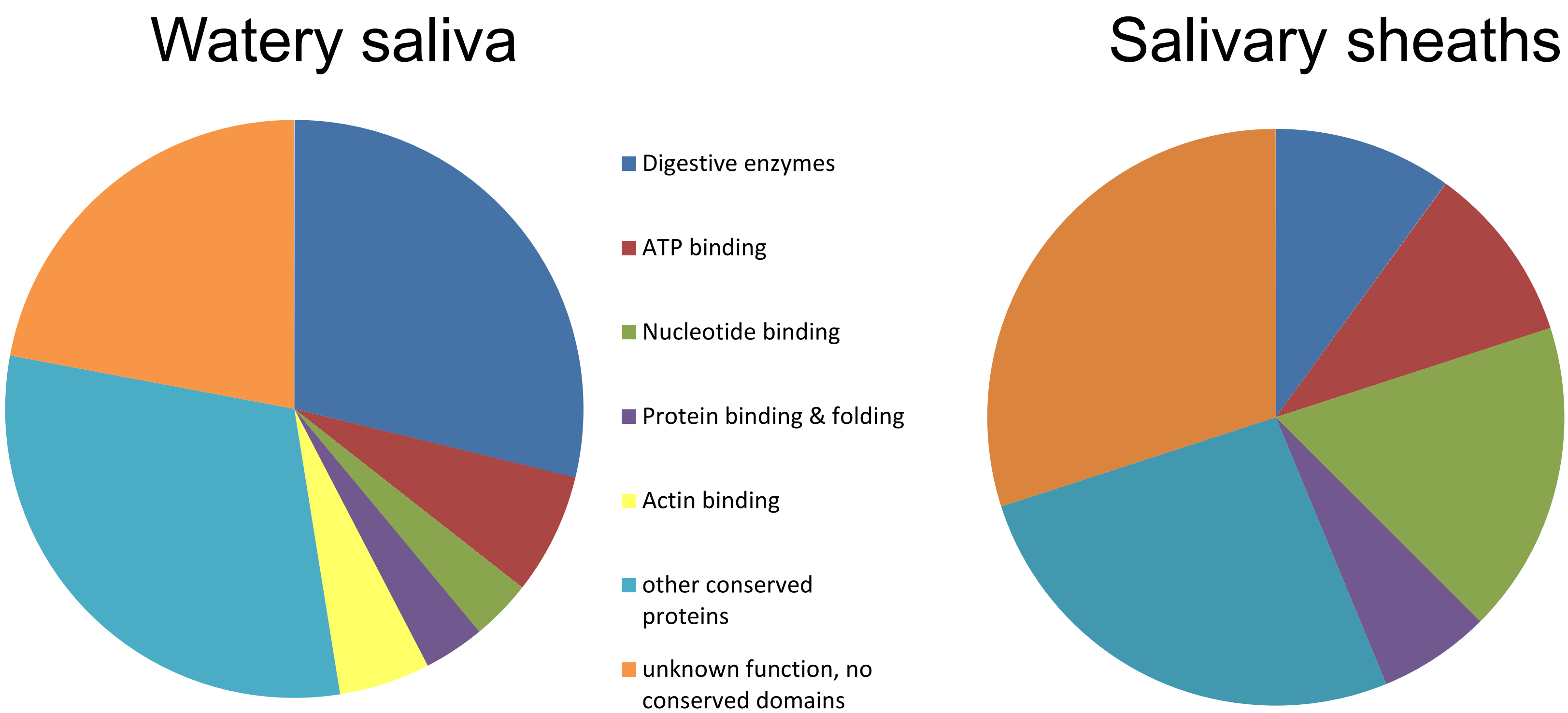
Principal Salivary Duct

Accessory Gland



SDS PAGE gel of BMSB watery saliva and salivary sheaths

Relative abundance of peptides identified by LC-MS/MS



Enzyme activities in BMSB watery saliva and salivary sheaths collected from tomatoes.

enzyme	watery saliva	salivary sheath
Amylase, µmole/min/mg	19 ± 0.04	440 ± 176
Peroxidase, mOD/min/mg	no activity	902 ± 309
Polyphenol oxidase	no activity	no activity
Glucose oxidase	no activity	no activity

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 amylase sequences:

  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

- 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

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Introduction

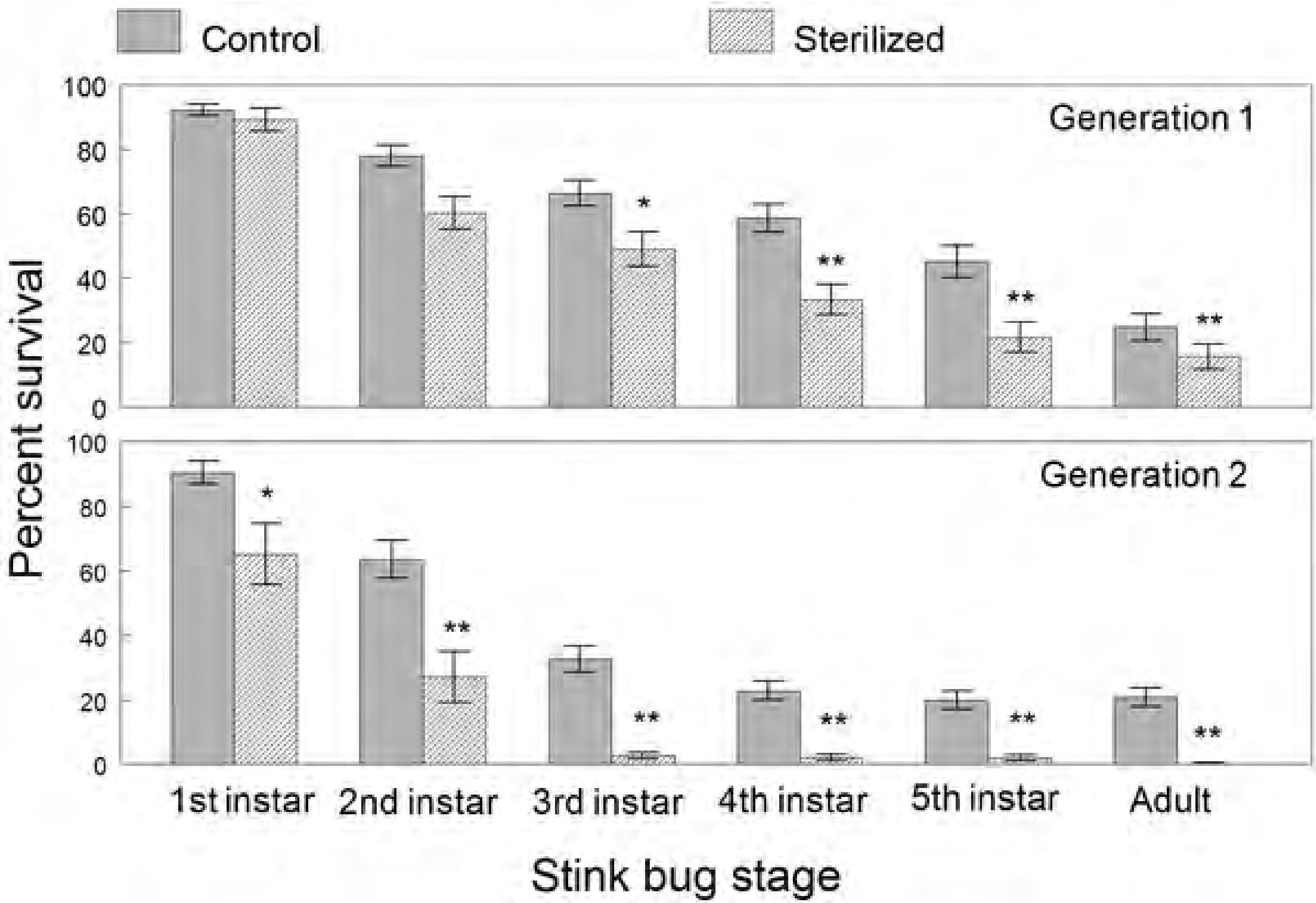
- BMSB symbiont was identified as a species of *Pantoea* in 2013 (DeLay 2013, unpublished) and then described and given the proposed name *Candidatus* "Pantoea carbekii" in 2014 (Bansal, Michel and Sabree 2014)


Probing behavior ~1 hour after hatch


Sucking behavior, ~1 hour after hatch

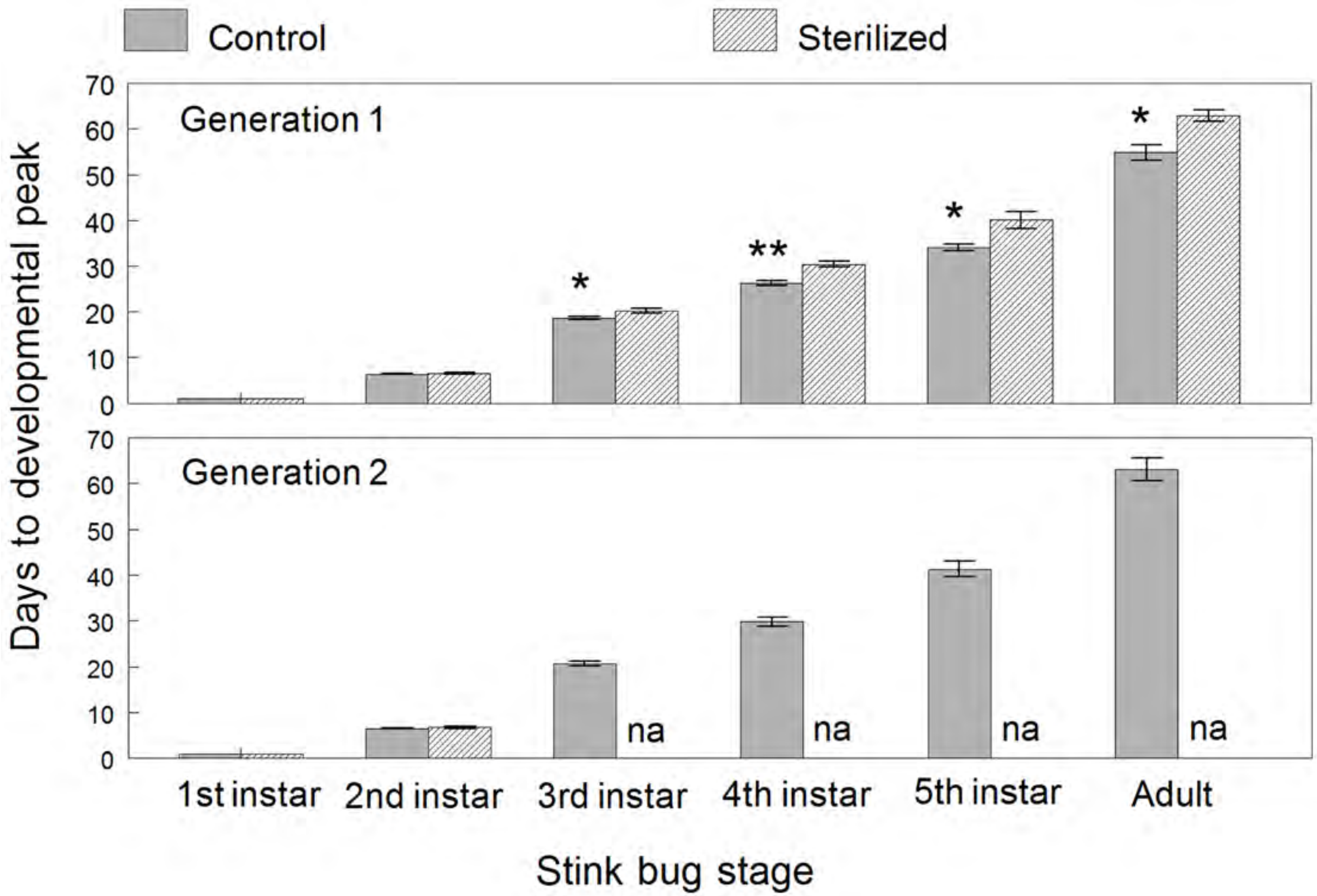

Clustering behavior of 1st instars (M. Raupp)

Results: Survival



Percent survival from egg hatch to the peak density of each developmental stage of *H. halys* during two successive generations.

Results: Development

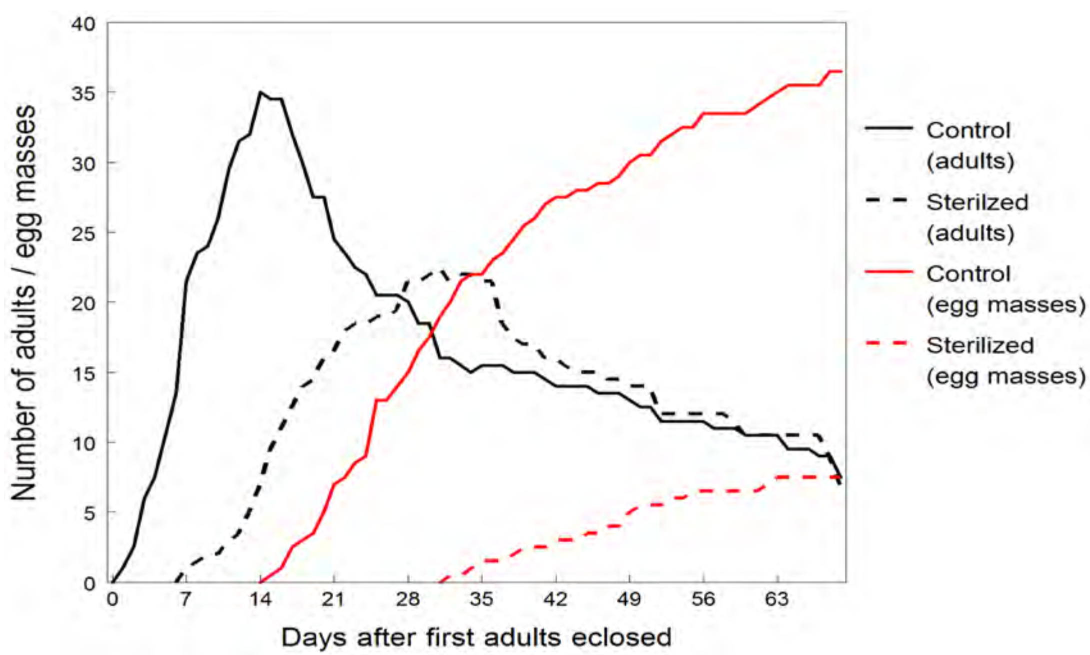


Development time expressed as the number of days from egg hatch required to reach peak density of each stage of *H. halys*.

Results: Fecundity

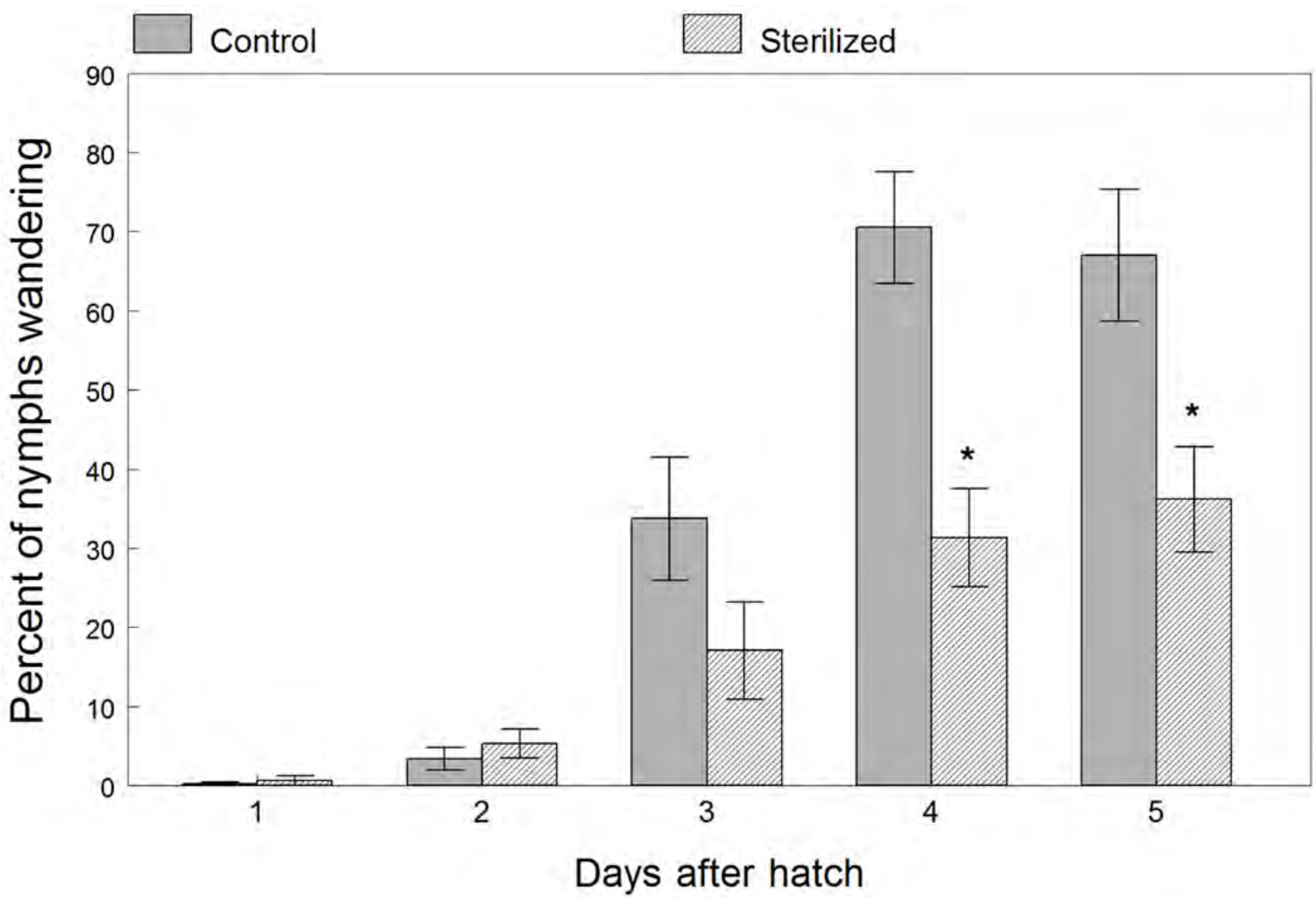
Parameter	*Control*	*Sterile*	*df*	*F*	*P*
Time to first egg mass (days)	15.5±1.5	25±0	-	-	-
Mean #egg masses/female	1.58±0.011	0.473±0.098	-	-	-
Mean # eggs/mass	27.77±0.01	17.13±2.05	1, 85.3	129.52	<.0001
Mean % hatch	94.43±1.30	64.95±9.46	1, 85	33.44	<.0001
Mean development time (days)	6.42±0.08	6.50±0.11	1, 83	0.18	0.6735

doi:10.1371/journal.pone.0090312.t004



Results: Behavior

- Significantly more nymphs wandering in control egg masses on days 4 and 5



Objectives

- Can we develop management strategies that target the symbiont on the egg mass surface to indirectly manage the brown marmorated stink bug?

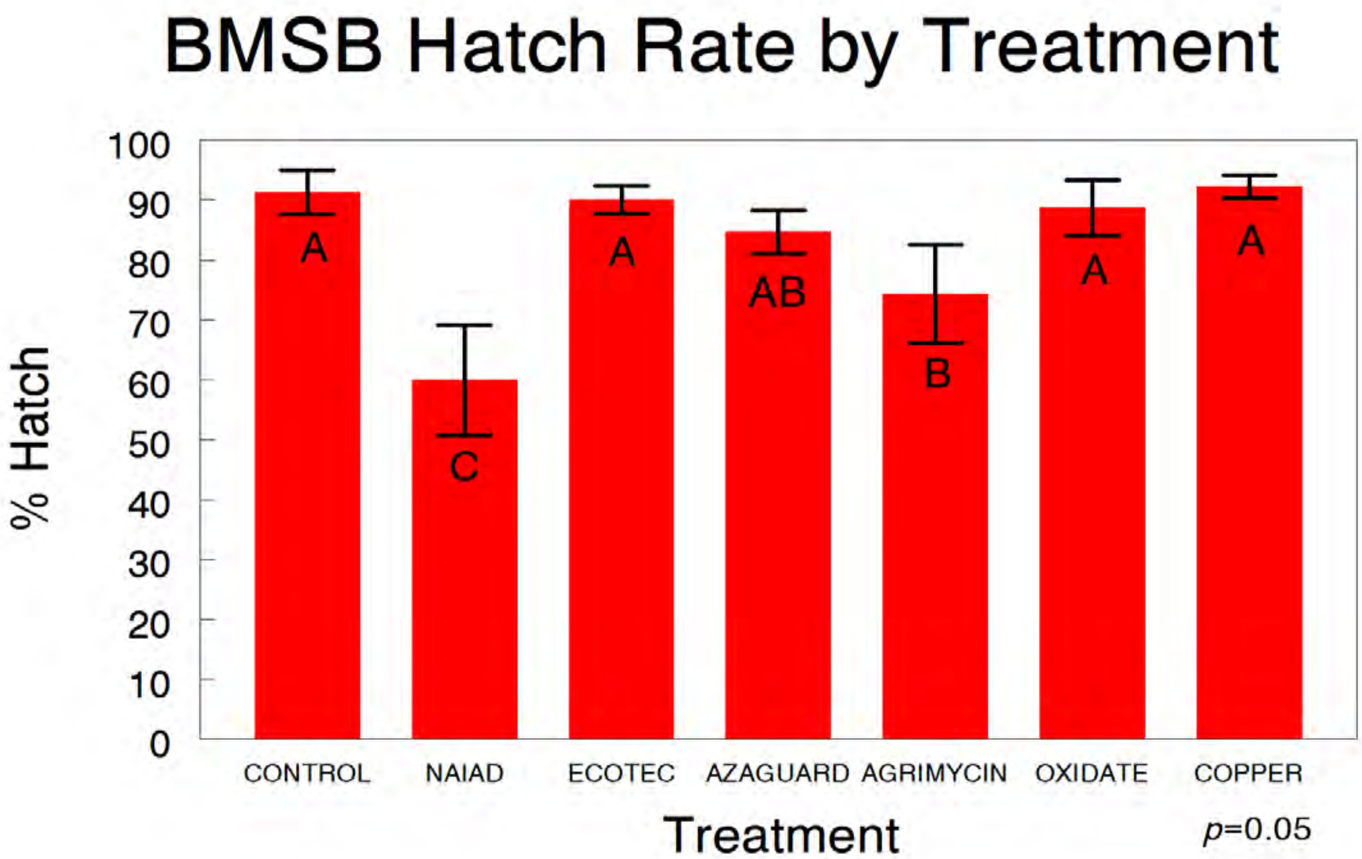
  - More specifically, can we use commercially available products to get adequate egg mass sterilization in the field?

Materials and Methods

Preliminary screening of products to test for direct and indirect effects on nymphs

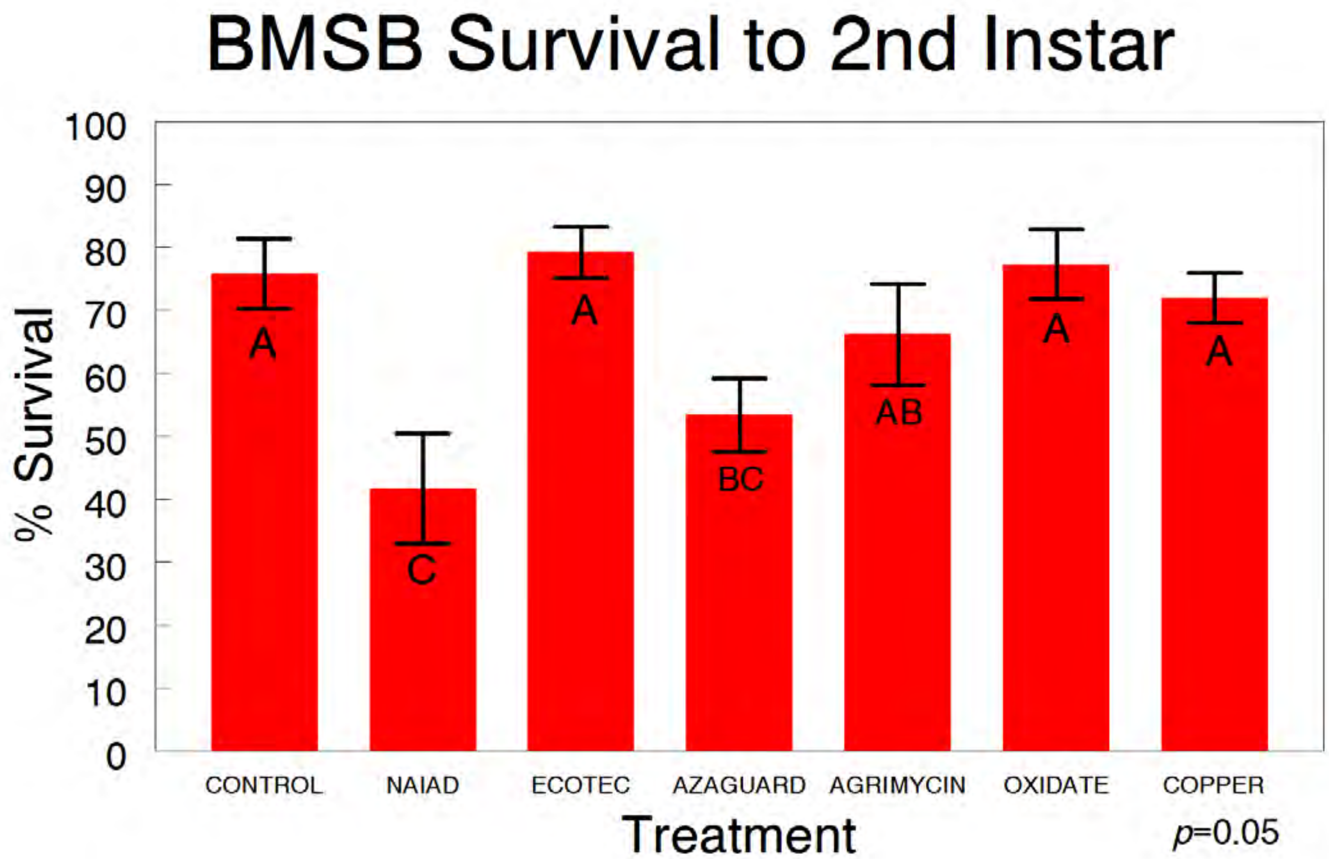
- 1 surfactant:
  - Naiad (Naiad Company, Inc.)
- 2 insecticides:
  - AzaGuard (BioSafe Systems LLC)
  - Ecotec (Brandt Consolidated, Inc.)
- 3 antimicrobials:
  - OxiDate 2.0 (BioSafe Systems LLC)
  - Agri-Mycin 17 (Nufarm Limited)
  - Liquid Copper Fungicide (Southern Agricultural Insecticides, Inc.)

Results: Hatch Rate



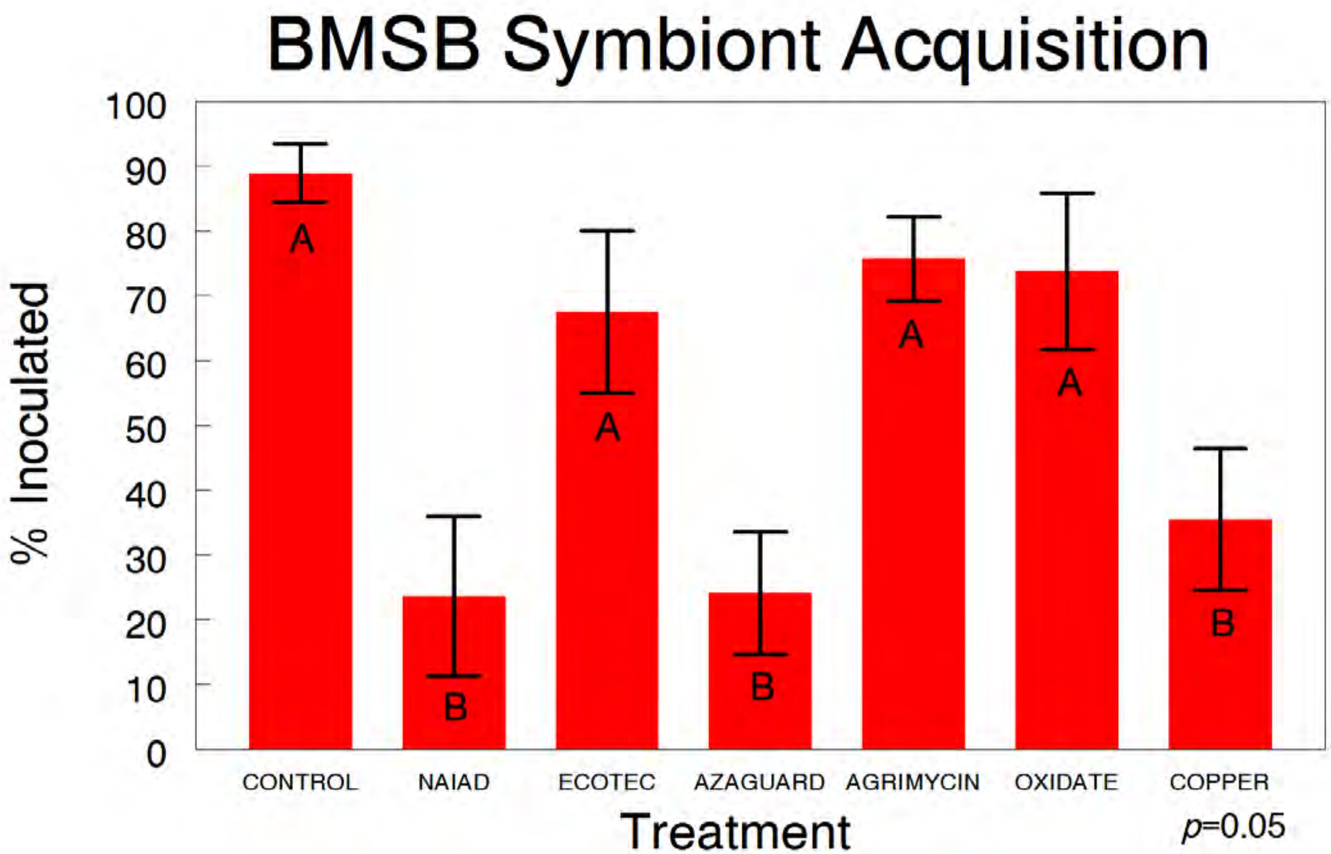
TREATMENT	# REPS
CONTROL	17
NAIAD	17
ECOTEC	17
AZAGUARD	17
AGRIMYCIN	17
OXIDATE	17
COPPER	17

Results: Survival



TREATMENT	# REPS
CONTROL	17
NAIAD	17
ECOTEC	17
AZAGUARD	17
AGRIMYCIN	17
OXIDATE	17
COPPER	17

Results: Symbiont Acquisition



TREATMENT	# REPS
CONTROL	9
NAIAD	8
ECOTEC	8
AZAGUARD	6
AGRIMYCIN	7
OXIDATE	8
COPPER	9

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Colony Rearing and Diapause

- Colony rearing is important for many reasons:
  - Life history studies
  - Parasitoid rearing
  - Bioassays/ toxicity studies
  - RNAi/ genetic research

Colony Rearing and Diapause

- Summary of findings
  - Optimal temperature and humidity
  - Mixed diet of proteins and carbohydrates
  - Diapause considerations
  - Issues with microsporidian infection