Examination of non-target effects of autodissemination approaches in lab and semi-field conditions

Sri Jyosthsna Kancharlapalli[1], Scott D. Longing[2] and Corey L. Brelsfoard[1]

[1]Department of Biological Sciences, Texas Tech University; [2]Department of Plant and Soil Science, Texas Tech University

INTRODUCTION

Autodissemination

- Autodissemination is a method of pesticide self-delivery, where insects will be used as delivery vehicles.
- Autodissemination approaches mainly target container breeding species such as *Aedes aegypti* and *Aedes albopictus*, which transmit pathogens responsible for dengue, chikungunya, and zika viruses.

Autodissemination Methods

- The two different methods of Autodissemination that are currently in use are Autodissemination Augmented by Males (ADAM) and placing a dissemination station (Refer to Figure 1 and Figure 2).

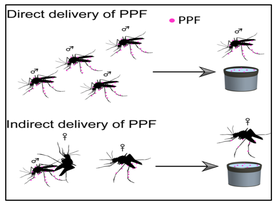

Figure 1. ADAM - Males deliver pyriproxyfen (PPF) directly by approaching breeding sites and indirectly through copulation

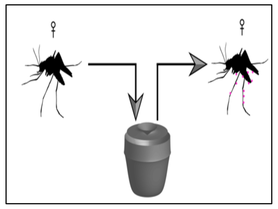

Figure 2. Dissemination stations – Gravid females enter the contaminated oviposition sites and disseminate to other resting sites

Advantages

- Less labor intensive as mosquitoes themselves act as dispersal agents.
- Need of relatively less active ingredient than conventional spraying techniques.

HYPOTHESIS

- *Ae. albopictus* males dusted with pyriproxyfen (PPF) will transfer PPF to the artificial nectar sources and indirectly contaminate the pollinators that share the common nectar source in the laboratory cages.
- When *Ae. albopictus* males are released into the semi-field cages along with females, painted lady butterflies and an autodissemination station, painted lady butterflies will be indirectly contaminated with the PPF.

RESEARCH OBJECTIVE

- Examine the nectar source and pollinator cross contamination in laboratory and Greenhouse cages

METHODS

Laboratory cage Experiments

- Fifteen adult male *Ae. albopictus* are dusted with PPF (Esteem 35 WP) mixed with fluorescent powder using handheld bellow duster and were released into the cages with different combination of eight honey bees or fifteen female mosquitoes or both in addition to artificial nectar source and oviposition cup. (Refer to Figure 6 (A) for the treatment cage types, insects and materials added to each cage).
- There were four replicates for each treatment and a control cage.
- Florescent imaging, mass spectrometry, survivorship and bioassays were conducted to confirm the hypothesis.
- Bioassays consists of four mosquito larvae in a 20mL glass scintillation vials and materials collected from cages (Refer to Figure 6 for bioassay substrates)

Greenhouse cage Experiments

- 250 dusted *Ae. albopictus* males were released into the greenhouse cage along with 30 painted lady butterflies (*Vanessa cardui*). Four natural nectar sources such as lavender and Russian sage plants including four artificial sucrose cups are placed in the cage.
- PPF imaging and bioassays were performed to confirm the direct transfer of PPF from male mosquitoes to the nectar source and indirect transfer to the butterflies.

FLOURESCENT IMAGING

Laboratory cage Experiments

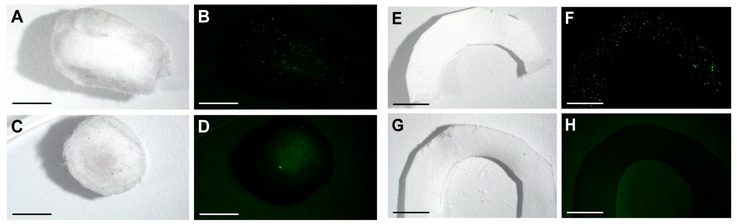

Figure 3 Images of cotton wick (CW) and filter paper (FP) ring of artificial nectar source (A) CW under visible light from the treatment cage (B) CW under Ultraviolet light (UV) (C) CW under visible light from the control cage (D) CW under UV from control cage (E) FP under visible light from the treatment cage (F) FP under Ultraviolet light (UV) (G) FP under visible light from the control cage (H) FP under UV from control cage.

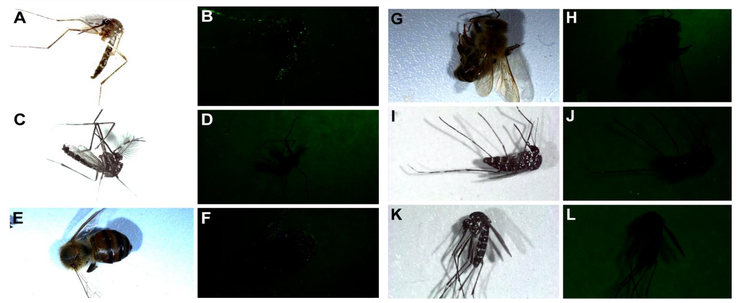

Figure 4 Images of *Ae. albopictus* males from treated cage (A) under visible light (B) under UV light; males from untreated cage (C) under visible light (D) under UV light; *Apis mellifera* from treated cage (E) under visible light (F) under UV light; *A. mellifera* from untreated cage (G) under visible light (H) under UV light; Ae albopictus females from treated cage (I) under visible light (J) under UV light; males from untreated cage (K) under visible light (L) under UV light.

Greenhouse cage Experiments

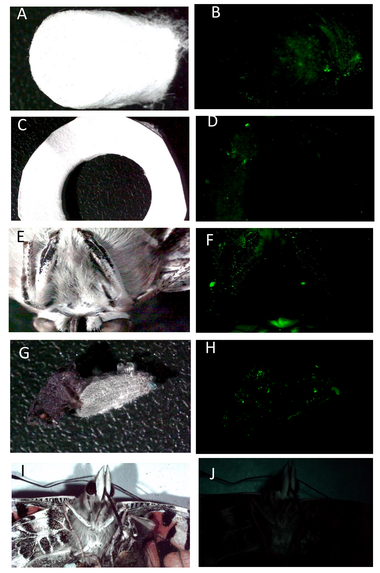

Figure 5 Images of artificial and natural nectar source materials. Cotton wick (A)under visible light (B) under UV light , Filter paper (C) under visible light (D) under UV light, Ventral side of butterfly (E) under visible light (F) under UV light, Russian sage flower (G) under visible Light (H) under UV light, Butterfly from untreated cage (I) under visible light (J) under UV light

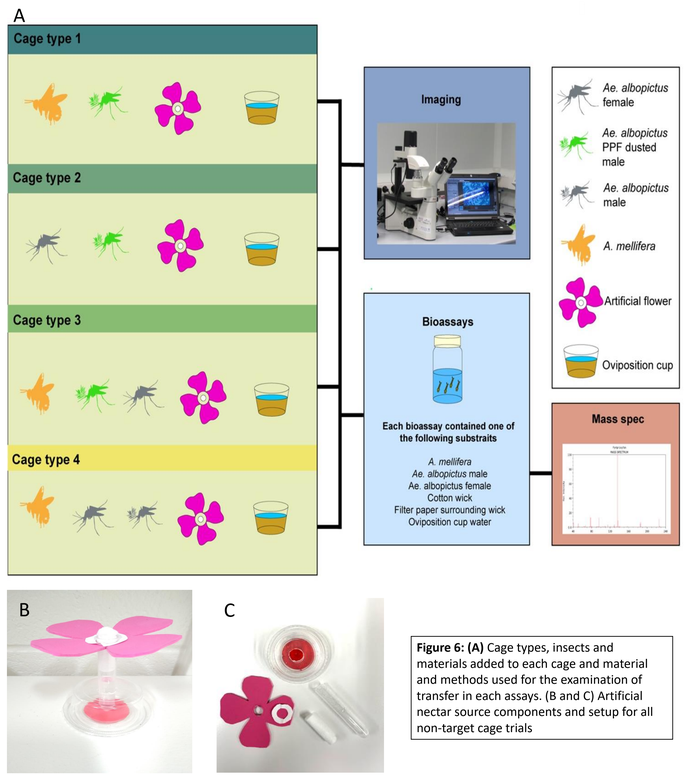

Figure 6: (A) Cage types, insects and materials added to each cage and material and methods used for the examination of transfer in each assays. (B and C) Artificial nectar source components and setup for all non-target cage trials

BIOASSAYS AND SURVIVORSHIP

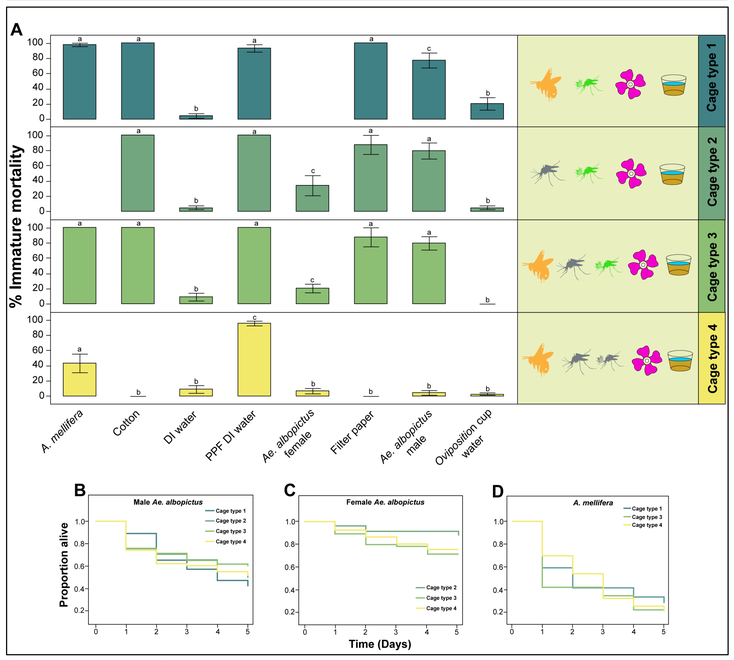

Figure 7. (A) Immature *Ae. albopictus* mortality in bioassays conducted using insects and materials collected from cage types 1-4. All data is represented by the mean immature mortality ± SEM. Letters above each bar represent significant differences as determined by t-tests ($P < 0.05$). (B) Survival plots of male *Ae. albopictus* (N = 4), (C) female *Ae. albopictus* (N = 4), and (D) *A. mellifera* (N = 3). All survival plots are mean numbers of surviving insects on each day for each cage type.

RESULTS

Laboratory cages

- Images of the artificial nectar source materials, bees, male, and female mosquitoes from the laboratory cages show the transfer of PPF directly and indirectly from PPF treated *Ae. albopictus* males (Refer to Figure 3 and 4) .
- Significant lethal effects were observed in the bioassays conducted with the nectar source materials suggesting the direct transfer of PPF from *Ae. albopictus* to nectar source. Bioassays conducted with the *A. mellifera* collected from all the cage types has shown significant mortality confirming the indirect transfer of PPF from nectar source to *A. mellifera* (Refer to Figure 7 (A) for the percentage immature mortality).
- Data suggests that there is no significant difference in the survival of males, females, and bees from treatment cages to the control cage (Refer to Figure 7 (B-D) for the survivorship curves).
- The amount of PPF was quantified by performing liquid-chromatography-mass spectrometry (LC-MS) analyses. Results suggest the presence of PPF on *A. mellifera* workers (0.49 ± 0.26 ng/ml).
- Transfer of PPF was also observed to nectar source materials. A mean concentration of PPF 0.19 ± 0.11 ng/ml and 0.19 ± 0.05 ng/ml was observed on the cotton wick and filter paper, respectively that made up the sucrose wick of the artificial nectar source.

Greenhouse cages

- Images in the Figure 5 confirm the transfer of PPF from the male mosquitoes to the butterflies, natural, and artificial nectar sources.
- 100% mortality was observed in the bioassays with the materials from the artificial nectar source such as cotton wick and filter paper.
- Significant difference in the immature mortality in bioassays was observed from the flowers collected from the treated cage.
- Middle leg and hind leg portions in bioassays caused significantly higher mortality than the wings.

DISCUSSION

1. *A. mellifera* are contaminated with the PPF in the presence of shared nectar source in cages with dusted male mosquitoes.
2. Cross contamination is observed irrespective of pollinator species as PPF was observed on *A. mellifera* in lab cages and *V. cardui* in greenhouse cages.
3. Presence of females didn't affect the transfer of PPF from males to nectar source and *A. mellifera.*
4. This study suggests risks to the pollinator species. So, it is important to consider these effects when designing the targeted application methodologies.

FUTURE WORK

- We continue to investigate non target effects of autodissemination in field and semi-field cages.

References

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