

Evaluation of the In2Care Mosquito Trap against *Culex quinquefasciatus* mosquitoes under semi-field conditions

Eva Buckner, Ana Romero-Weaver, Natalie Kendziorski, Sierra Schluep, Rebecca Zimler, & Shawna Bellamy

University of Florida, Institute of Food & Agricultural Sciences, Department of Entomology & Nematology, Florida Medical Entomology Laboratory (UF/IFAS FMEL), Vero Beach, FL

Why the In2Care Mosquito Trap?

- Public health importance of *Ae. aegypti* and *Ae. albopictus*
 - Vectors of CHIKV, DENV, YFV, ZIKV
- Without vaccines for most *Aedes*-borne viral diseases, disease prevention = vector control
- However, traditional control methods may fall short against *Ae. aegypti* and *Ae. albopictus*
 - Immature development in small, cryptic containers difficult to detect or reach with larvicides



- Adulticide resistance documented in populations worldwide



- Need for additional control tools due to potentially limited success of strategies

What is the In2Care Mosquito Trap?

- In2Care Mosquito Trap designed to be attractive to skip-ovipositing *Aedes aegypti* & *Ae. albopictus*
- Combines pyriproxyfen (PPF) autodissemination with entomopathogenic fungus *Beauveria bassiana* to reduce emergence of *Aedes* mosquitoes from larval habitats & subsequently kill contaminated adult females



My history with the In2Care trap

- Senior Research Biologist at Manatee County Mosquito Control District in Palmetto, FL from 2015-2018
 - In2Care trap semi-field trials against *Ae. aegypti* and *Ae. albopictus*
 - In2Care trap field study against *Ae. aegypti*
- Assistant Professor and State Extension Specialist at UF/IFAS FMEL from 2018-present
 - In2Care trap semi-field trials against *Cx. quinquefasciatus*

Journal of the American Mosquito Control Association, 33(3):193–199, 2017
Copyright © 2017 by The American Mosquito Control Association, Inc.

EVALUATING THE VECTOR CONTROL POTENTIAL OF THE IN2CARE® MOSQUITO TRAP AGAINST *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* UNDER SEMIFIELD CONDITIONS IN MANATEE COUNTY, FLORIDA

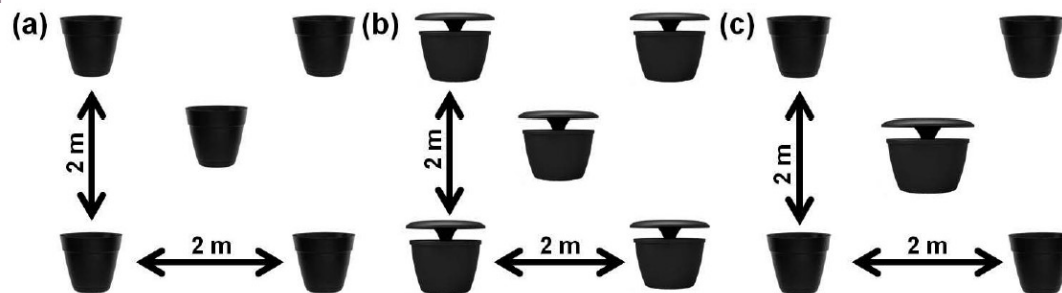
EVA A. BUCKNER, KATIE F. WILLIAMS, AMBYR L. MARSICANO, MARK D. LATHAM AND
CHRISTOPHER R. LESSER

Manatee County Mosquito Control District, 2317 2nd Avenue W, Palmetto, FL 34221



Semifield trials against *Ae. aegypti* & *Ae. albopictus*

- Objectives were to assess trap:
 - Attractiveness
 - PPF autodissemination
 - Adulticidal impacts



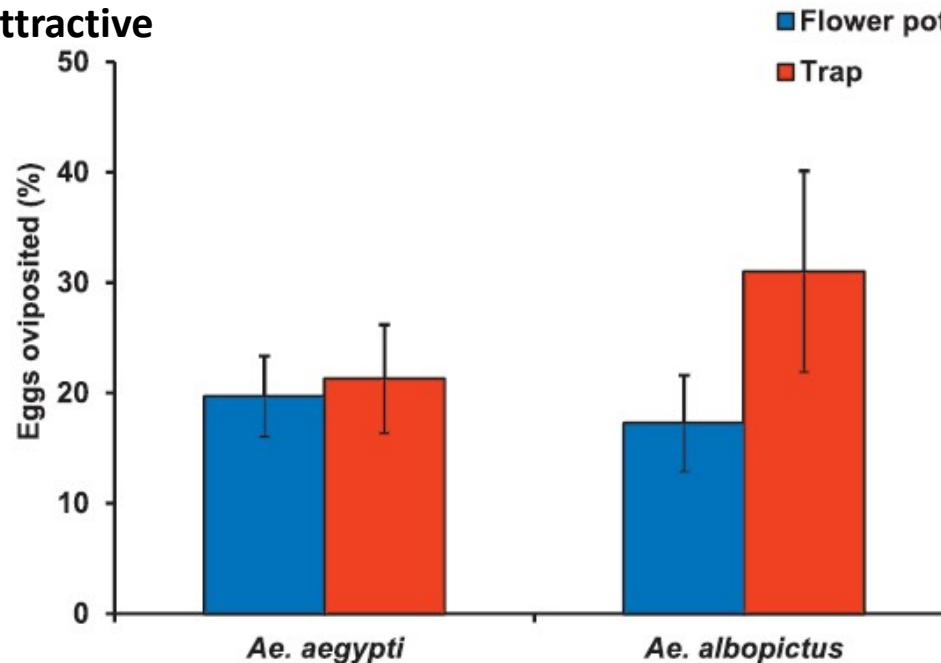
Schematics of experiments. (a) Negative control experiment with 5 flowerpots. (b) Positive control experiment with 5 In2Caret mosquito traps. (c) Treatment (dissemination) experiment with 1 In2Care mosquito trap and 4 flowerpots

Treatment (dissemination) experiment setup in screen room



Trap attractiveness

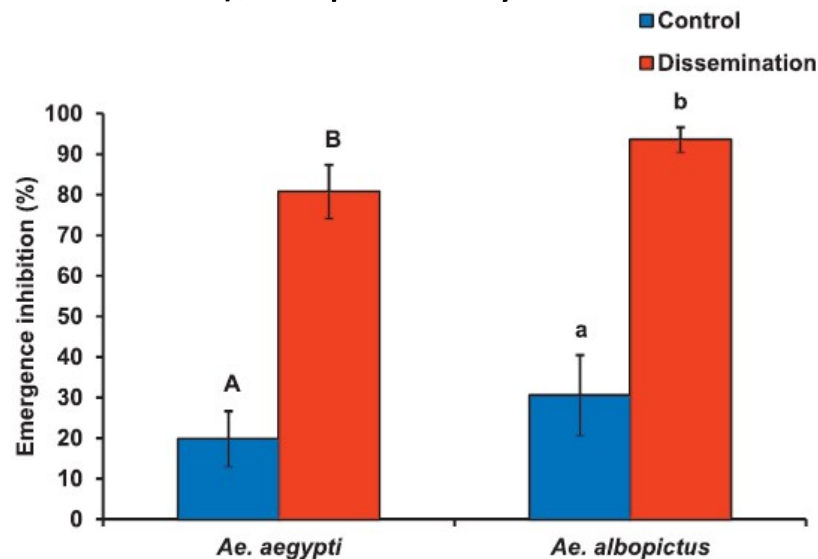
- In treatment experiments
 - Mean % of eggs laid by *Ae. aegypti* and *Ae. albopictus* in In2Care trap vs. surrounding flowerpots did not significantly differ
 - Trap was attractive



Mean percentage of eggs (\pm SE) laid in flowerpots versus the In2Care mosquito trap by *Ae. aegypti* and *Ae. albopictus* in treatment experiment replicates, which did not significantly differ ($P = 0.700$ and $P = 0.135$, respectively).

PPF autodissemination

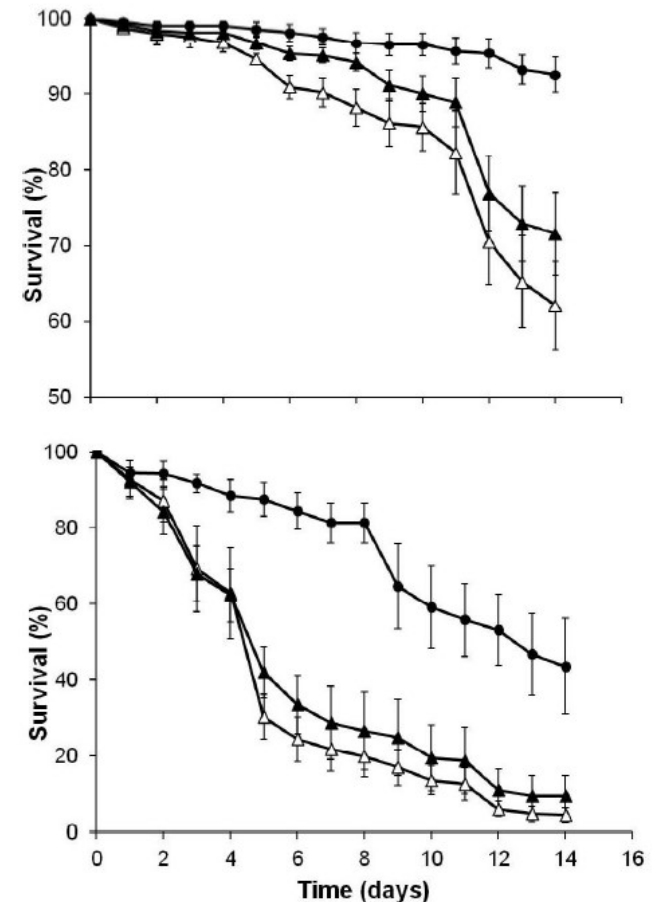
- Successful PPF autodissemination was confirmed for both *Ae. aegypti* and *Ae. albopictus*
 - *Ae. aegypti* & *Ae. albopictus* treatment (dissemination) emergence inhibition (81% & 94%) significantly higher than control emergence inhibition (20% and 31%), respectively



Mean (\pm SE) adult emergence inhibition rates of *Ae. aegypti* and *Ae. albopictus* larvae (n=20) added to 5 flowerpots in control experiment replicates versus 4 flowerpots placed near an In2Care mosquito trap in dissemination experiment replicates. Different letters above bars indicate significant differences ($P = 0.0002$ for *Ae. aegypti* and $P = 0.0003$ for *Ae. albopictus*)

Adulticidal impacts

- *Ae. aegypti* & *Ae. albopictus* survivorship was significantly reduced when In2Care mosquito traps with *B. bassiana* spore-treated gauze strips were deployed in the screen rooms



Mean (\pm SE) cumulative percentage of survival of recaptured *Ae. aegypti* (top) and *Ae. albopictus* (bottom) mosquito cohorts released inside semifield screen rooms with 5 flowerpots (closed circles), 5 In2Care mosquito traps (open triangles), or 1 In2Care mosquito trap and 4 flowerpots (closed triangles)

Journal of the American Mosquito Control Association, 37(4):000–000, 2021
Copyright © 2021 by The American Mosquito Control Association, Inc.

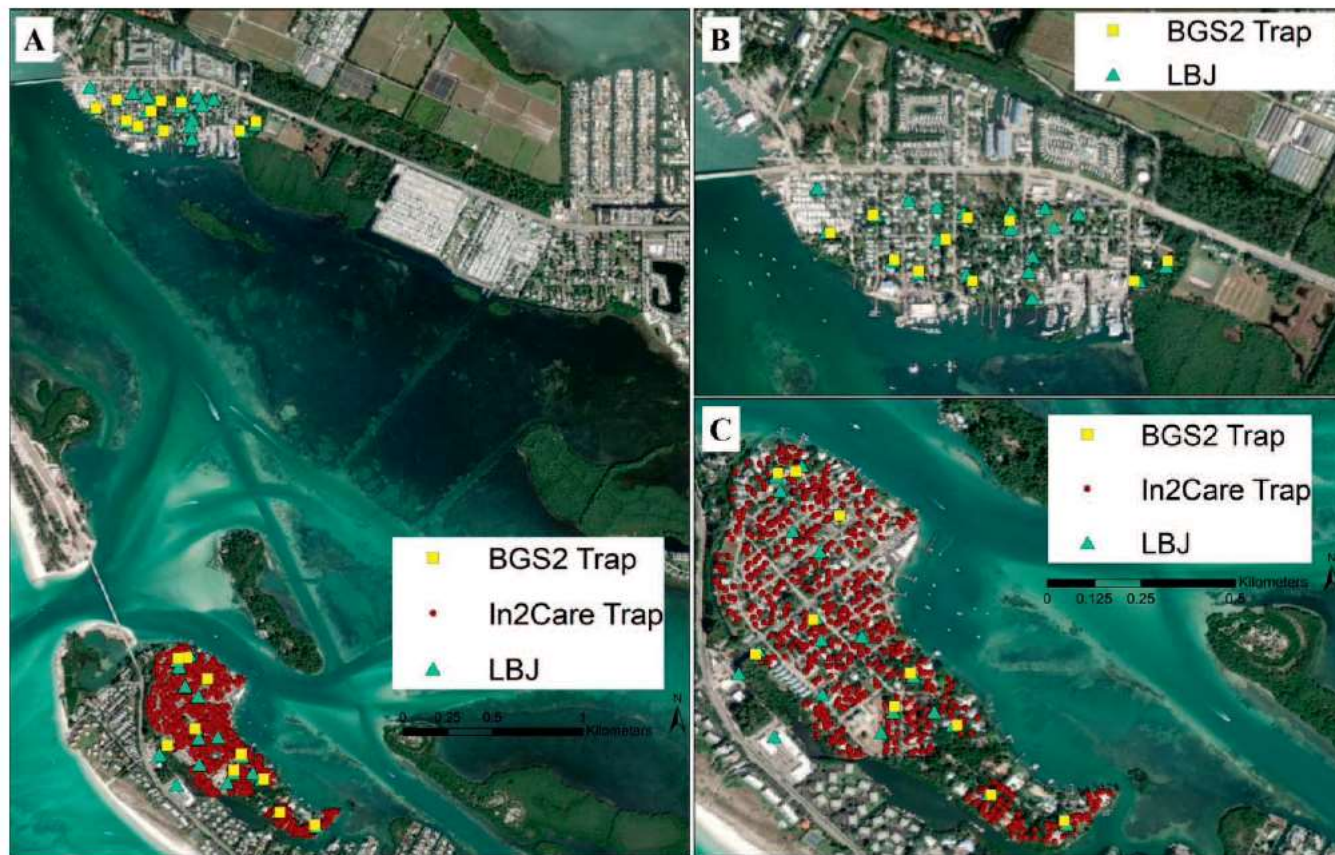
A FIELD EFFICACY EVALUATION OF IN2CARE MOSQUITO TRAPS IN COMPARISON WITH ROUTINE INTEGRATED VECTOR MANAGEMENT AT REDUCING *Aedes Aegypti*

EVA A. BUCKNER,¹ KATIE F. WILLIAMS,² SAMANTHA RAMIREZ,³ CONSTANCE DARRISAW,⁴
JULIANA M. CARRILLO,⁵ MARK D. LATHAM³ AND CHRISTOPHER R. LESSER³

Where do In2Care Mosquito Traps fit into operational mosquito control?

- Can they be used as alternative to conventional *Ae. aegypti* reduction techniques?
- Compare **efficacy** of In2Care Mosquito Traps only to reduce *Ae. aegypti* at a site (In2Care Trap site) to using conventional *Ae. aegypti* reduction techniques at similar site (IVM site)
 - Source reduction
 - Ground and aerial larviciding
 - Ground and aerial adulticiding

Methods



Maps of the integrated vector management (IVM) and In2Care Mosquito Trap (In2Care Trap) sites. (A) IVM and In2Care Trap sites in relation to each other; (B) Close-up of IVM site showing placement of BG-Sentinel 2 (BGS2) traps and little black jars (LBJ); (C) Close-up of In2Care Trap site showing placement of BGS2 traps, LBJ, and In2CareTraps.

Methods

- *Ae. aegypti* population data collected weekly at both sites during baseline & treatment periods:
 - # of eggs & larvae in 20 little black jars
 - # of adults collected by 10 BG-Sentinel 2 traps run once a week
- Baseline: 4 weeks before traps added to In2Care site
- Treatment: 4+ months after traps added to In2Care site



Little black jar



BG-Sentinel 2 trap

Methods

Table 1. Total time spent and transportation mode used for each mosquito control activity in the In2Care Mosquito Trap (In2Care Trap) and integrated vector management (IVM) sites during trial.

Site	Activity	Total time spent	Transportation mode
In2Care Trap	Installing traps	300 h	On foot
In2Care Trap	Trap maintenance	480 h	On foot
IVM	Daily inspections, source reduction, larviciding	150 h	On foot
IVM	Citizen service requests	3.5 h	On foot
IVM	GULV ¹	2.5 h	Truck
IVM	AULV ²	20 min	Helicopter

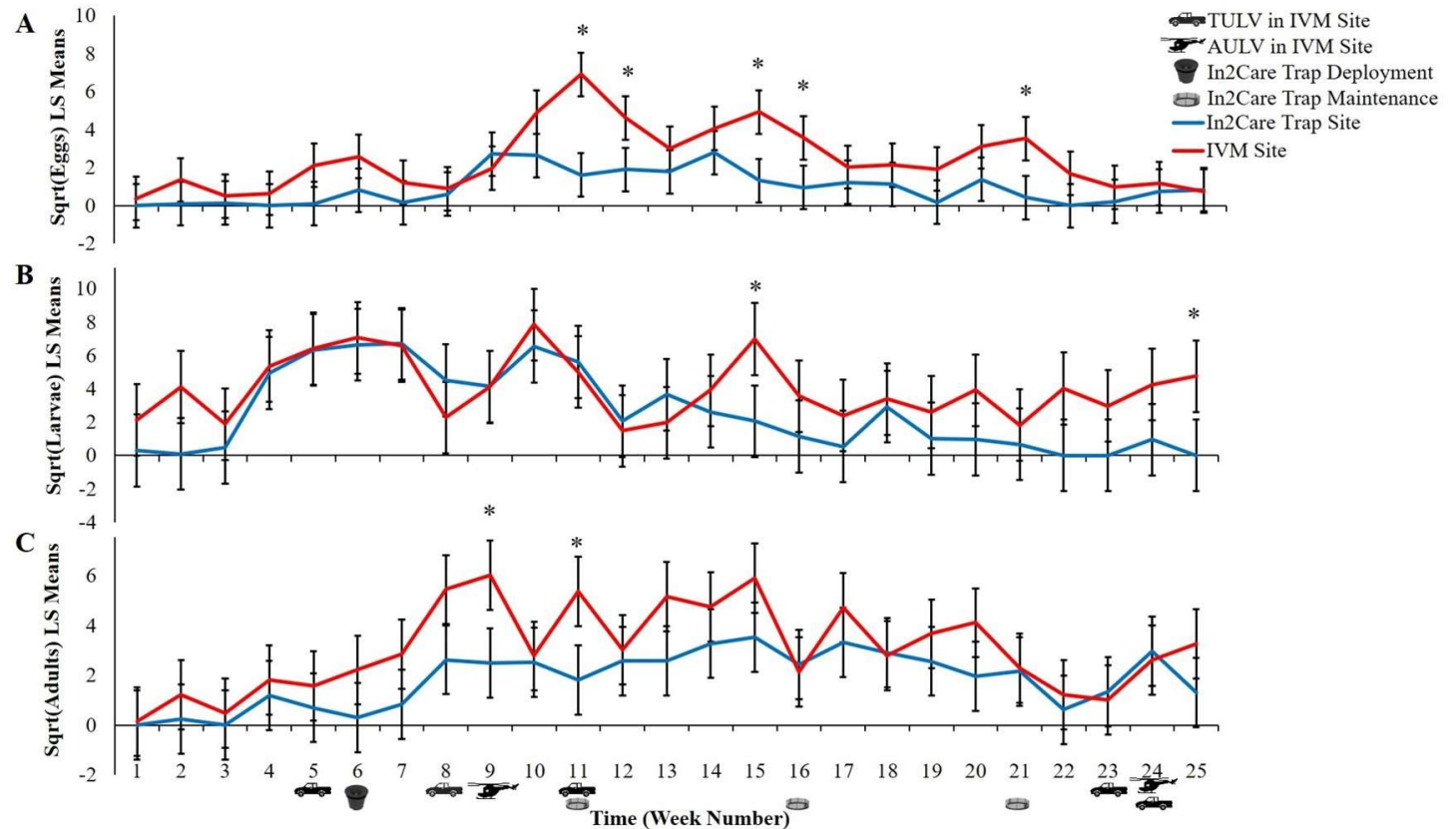
¹ Ground ultra-low-volume spray mission.

² Aerial ultra-low-volume spray mission.

Results

- Overall, the difference between treatments (IVM vs. In2Care Trap) was only statistically significant for eggs and larvae ($P < 0.05$ for eggs and larvae and $P > 0.05$ for adults), the use of In2Care Traps alone resulted in 60%, 57%, and 57% fewer eggs, larvae, and adults, respectively, collected from that site compared to the IVM site

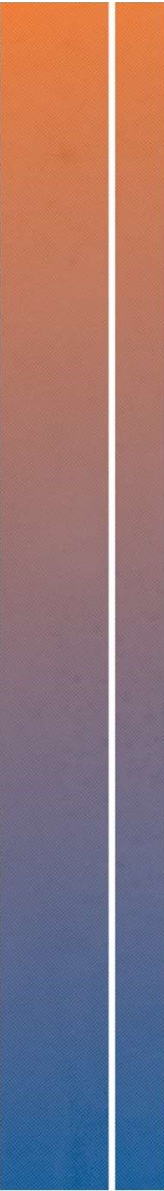
Results



Aedes aegypti weekly mean density in the In2Care Mosquito Trap (In2Care Trap) and integrated vector management (IVM) sites. (A) Mosquito eggs collected in little black jars (LBJ); (B) Mosquito larvae collected in LBJ; (C) Mosquito adults collected in BG-Sentinel 2 traps. Square-root-transformed weekly least squares (LS) means are shown. Asterisk indicates weeks when LS means significantly differed between sites. The timing of aerial and ground ultra-low volume adulticide applications in the IVM site is indicated by the black truck or helicopter symbol, respectively. The timing of In2Care Trap deployment is indicated by the black In2Care Trap symbol, and the timing of In2Care Trap maintenance in the In2Care Trap site is indicated by the black In2Care Trap floater symbol. (D) Trial timeline.

Results/Discussion

- In2Care Trap deployment and maintenance were more time consuming and labor intensive than the IVM strategy
- Using In2Care Traps alone as control method for large areas (e.g., >20 ha) may be less practical for control programs with the capacity to conduct ground and aerial larviciding and adulticiding.
- In2Care Traps are effective at suppressing *Ae. aegypti*
 - **Have the most potential for use in areas without sophisticated control programs and within IVM programs**
 - Could particularly be useful in small-sized hotspots or target areas within IVM program



Evaluation of the In2Care Mosquito Trap against *Culex quinquefasciatus* mosquitoes under semi-field conditions

Eva Buckner, Ana Romero-Weaver, Natalie Kendzierski, Sierra Schluep, Rebecca Zimler, & Shawna Bellamy

University of Florida, Institute of Food & Agricultural Sciences, Department of Entomology & Nematology, Florida Medical Entomology Laboratory (UF/IFAS FMEL), Vero Beach, FL

Introduction

- Observations made by Su et al. 2020 & Buckner et al. 2021 during field experiments suggest that In2Care Traps may be attractive oviposition & resting sites for *Culex quinquefasciatus*
- *Cx. quinquefasciatus* does not exhibit skip-oviposition & lays eggs all at once in raft
 - More selective in choosing oviposition site
 - May visit several potential sites before ovipositing
- Mbare et al. 2014 demonstrated in lab setting that gravid *Cx. quinquefasciatus* adult females can transfer lethal concentrations of PPF to oviposition sites
- **Due to public health importance of insecticide-resistant *Cx. quinquefasciatus*, we conducted semi-field experiments to determine if In2Care Traps could potentially be effective against species**



Methods

Experiment Setup

Control

- 4 flowerpots surrounding flowerpot
- 5 replicates



20 *Culex quinquefasciatus* larvae

Treatment

- 4 flowerpots surrounding In2Care Trap
- 5 replicates



Release-recapture procedures

50 gravid adult *Culex quinquefasciatus* mosquitoes released

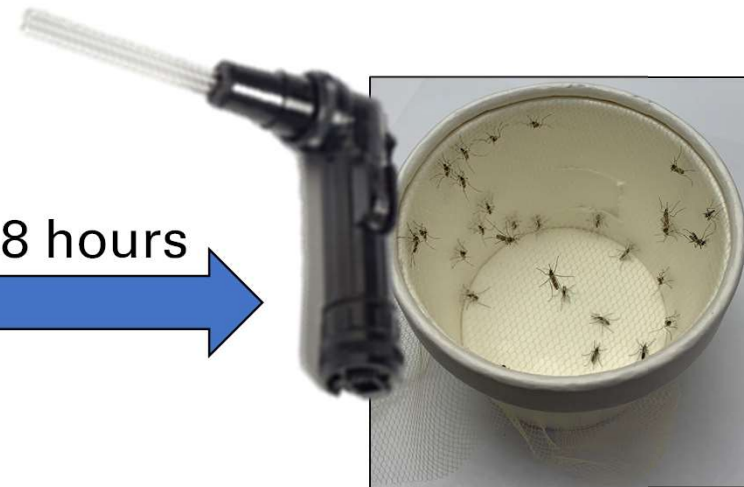
Control



Treatment



48 hours



Trap attractiveness



- Mean % rafts laid in In2Care Trap compared to mean % rafts laid in flowerpot in treatment experiment

PPF autodissemination

- Mean % adult emergence inhibition from flowerpots in control replicates compared to mean % adult emergence inhibition from flowerpots surrounding In2Care trap in treatment replicates



versus



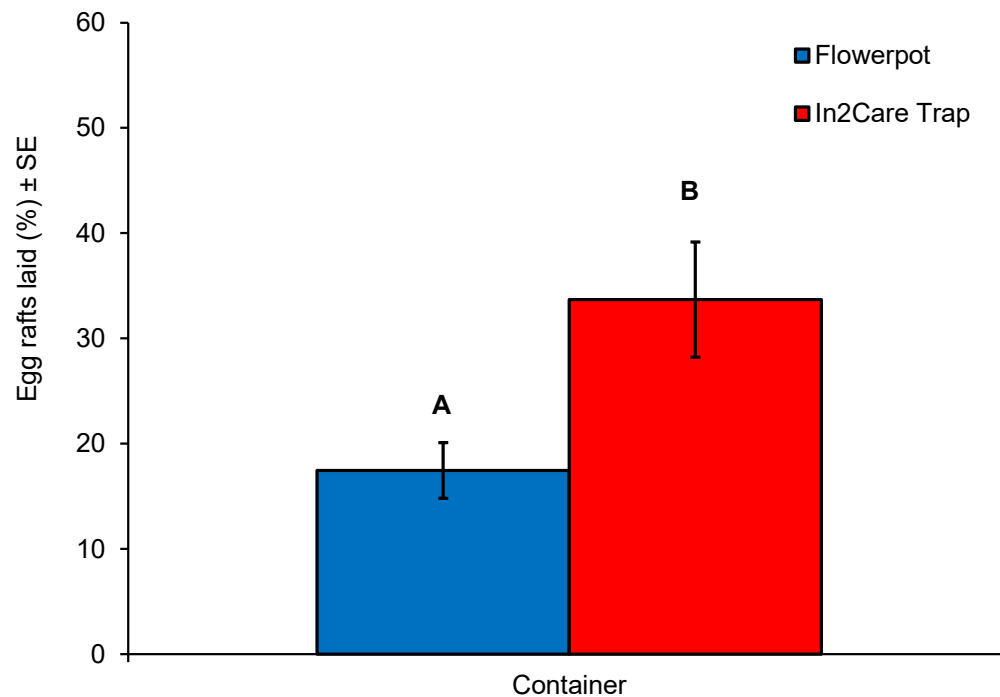
Effect of *Beauveria bassiana* spores on adult survivorship





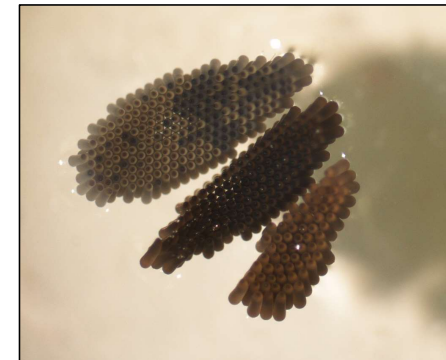
Results

Trap attractiveness

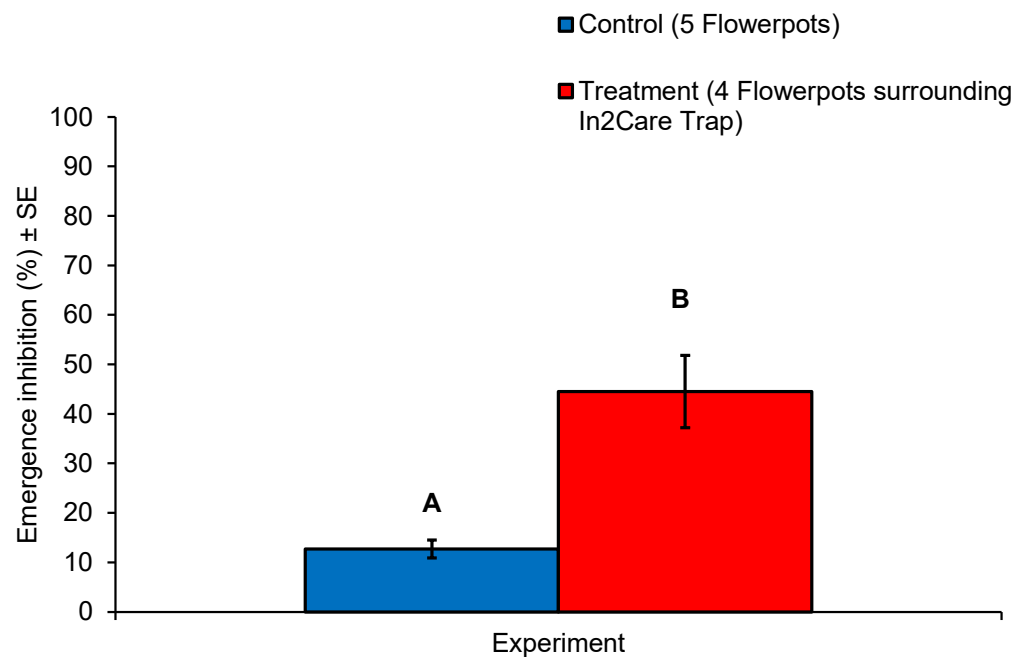


Mean percentage of egg rafts laid (\pm SE) in flowerpot (blue) versus In2Care Trap (red) by *Cx. quinquefasciatus* adults in treatment replicates. Different letters above bars indicate significant differences ($P=0.016$).

- In treatment replicates, gravid *Cx. quinquefasciatus* females laid significantly higher percentage of egg rafts in In2Care Trap ($34 \pm 5\%$) compared to flowerpot ($18 \pm 3\%$) ($P=0.016$)



PPF autodissemination

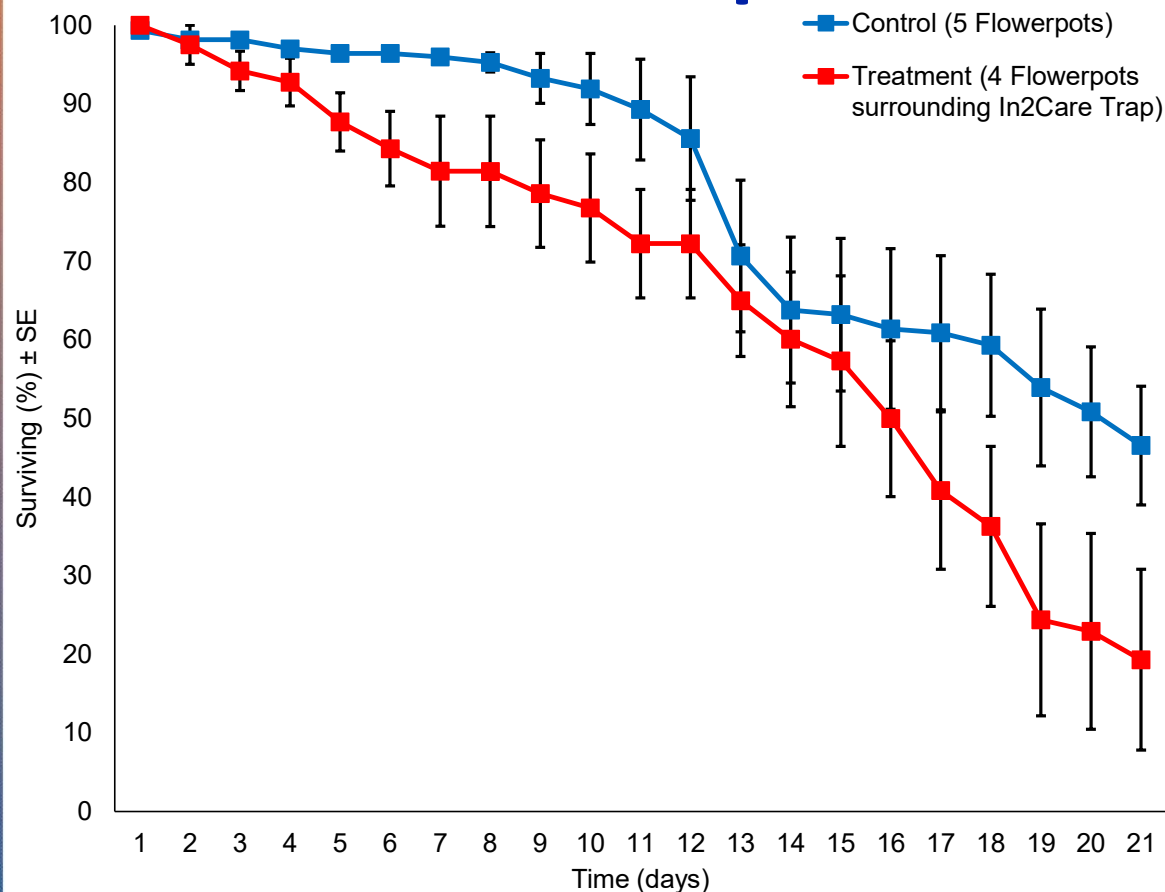


Mean (\pm SE) adult emergence inhibition rates of *Cx. quinquefasciatus* larvae ($n=20$) added to 5 flowerpots in control replicates (blue) versus 4 flowerpots surrounding an In2Care Trap in treatment replicates (red). Different letters above bars indicate significant differences ($P<0.001$).

- Treatment emergence inhibition ($45 \pm 7\%$) significantly higher than control emergence inhibition ($13 \pm 2\%$) ($P<0.001$)
- Observed PPF-specific pupicidal effect (black uncurled dead pupae) in treatment experiments



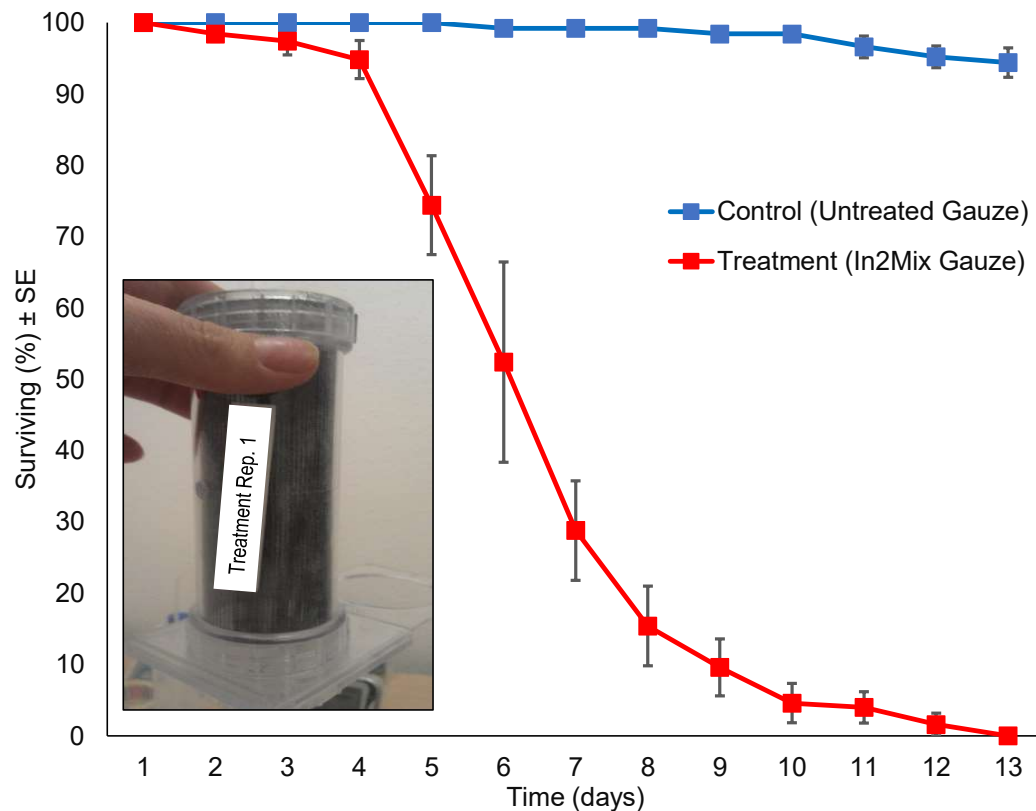
Effect of *Beauveria bassiana* spores on adult survivorship



Mean (\pm SE) cumulative % survival of recaptured adult *Culex quinquefasciatus* cohorts released inside tents with 5 flowerpots (blue) or 1 In2Care Trap and 4 flowerpots (red).

- No significant difference in adult survivorship ($P=0.131$)
- Most likely influenced by low adult recapture rate in treatment replicates
 - 88% = avg. recapture rate for control
 - 30% = avg. recapture rate for treatment

Effect of *Beauveria bassiana* spores on adult survivorship after forced exposure



- Treatment survivorship significantly lower than control survivorship ($P < 0.001$)

Mean (\pm SE) cumulative % survival of adult *Culex quinquefasciatus* cohorts (N=25, n=5) after 3-min exposure to clean netting (blue) or In2Mix-treated netting used in In2Care Traps (red) in WHO bioassay tubes.

Summary

- In2Care Trap was attractive oviposition site for gravid *Cx. quinquefasciatus* females
- Adult *Cx. quinquefasciatus* females successfully autodisseminated PPF from In2Care Trap to surrounding flowerpots
- Exposure to *Beauveria bassiana* spores during forced bioassays significantly reduced survivorship of *Cx. quinquefasciatus* adults
- In2Care Trap may be effective control tool against *Cx. quinquefasciatus*
 - Field evaluations needed

Thanks to...

- In2Care B.V. for providing In2Care Traps & In2Mix refills
- MCMCD & residents of Manatee County, FL
- Jason Conrad for invitation to present
- Mark Blackmore and Danny Mead

Questions?

- Email me at eva.buckner@ufl.edu

References Cited

- Buckner EA, Williams KF, Marsicano AL, Latham MD, Lesser CR. 2017. Evaluating the vector control potential of the In2Care® Mosquito Trap against *Aedes aegypti* and *Aedes albopictus* under semifield conditions in Manatee County, Florida. J Am Mosq Control Assoc 33:193-199.
- Buckner EA, Williams KF, Ramirez S, Darrisaw C, Carrillo JM, Latham MD, Lesser CR. 2021. A field efficacy evaluation of In2Care Mosquito Traps in comparison with routine integrated vector management at reducing *Aedes aegypti* mosquitoes. J Am Mosq Control Association. Accepted.
- Mbare O, Lindsay SW, Fillinger U. 2014. Pyriproxyfen for mosquito control: female sterilization or horizontal transfer to oviposition substrates by *Anopheles gambiae* sensu stricto and *Culex quinquefasciatus*. Parasit Vectors 7:280.
- Su T, Mullens P, Thieme J, Melgoza A, Real R, Brown MQ. 2020. Deployment and fact analysis of the In2Care® Mosquito Trap, a novel tool for controlling invasive *Aedes* species. J Am Mosq Control Assoc 36:167-174.