

AMCARF 2021 Project Annual Report

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Report Type: Annual Report

Project Title: “Modified Attractive Toxic Sugar Baits for Aedes Vectors”

AMCARF project number: 2021-01

Project Cost: \$51,618

Project Leader: Dr. Jason Pitts

Collaborators: (Include cooperating laboratories and AMCARF supported personnel and percent effort)

Heidi Lindsley, PhD student. 100% effort (continuing through May, 2022)

Melissa Noreña, undergraduate student. 10 hours per week (continuing through May, 2022)

Harris County Public Health, Vector Control Division. Collaborating institution in year 2.

Project Objectives:

- Develop odor blends that attract *Ae. aegypti* & *Ae. albopictus* in the context of sugar feeding.
- Identify GRAS compounds that reduce mosquito lifespan, especially at sublethal concentrations.
- Conduct field trials of ATSB attraction in McLennan County (Waco) and Harris County (Houston), Texas

Total Project Progress:

We have initiated trials related to objective 2: *Identify GRAS compounds that reduce mosquito lifespan, especially at sublethal concentrations.* Specifically, we have tested four diols for their effects on survivorship in *Aedes aegypti*. Each of these compounds shows lethality at low concentrations when mixed with 5-10% sucrose and presented to adult mosquitoes *ad libitum*. Importantly, this lethality is also evident when allowing mosquitoes to access to the compounds for only 24 hours, which we predict mimics a single feeding paradigm that is more likely to occur in the context of an ATSB in a natural setting. As of June, we have a full-time graduate student and a part-time undergraduate student working on this project and we have made rapid progress on testing additional compounds. We also initiated dual choice feeding assays in the Fall semester that will continue in 2022.

Key Research Accomplishments:

- Extended our previous studies of effects of diols and sugar substitutes on survivorship in *Ae. aegypti*.
- Demonstrated dosage effects for 1,2-Propanediol against *Ae. aegypti*.
- Observed reduced life span of adult *Ae. aegypti* and *Ae. albopictus* with 24-hour access (single feeding) to 1,2-Propanediol.
- Discovered sex-dependent differences in survivorship in both *ad libitum* and single-feeding paradigms.
- Initiated dual choice feeding assays.

Reportable Outcomes:

Describe major outputs including for example papers, inventions filed and patents issued, or new mosquito control guidance or practices.

We are drafting a manuscript describing the effects of 1,2-Propanediol on *Ae. aegypti* and *Ae. albopictus* survivorship. We expect to submit this manuscript to a peer-reviewed journal early in 2022. Additionally, we expect to submit a second manuscript describing the effects of sugar substitutes on survivorship in *Ae. aegypti*.

Describe how the project has advanced the field of mosquito control scientifically.

Utilizing new compounds that have lethal or sub-lethal (life-shortening) effects in *Ae. aegypti/Ae. albopictus* is desirable, especially as they may help reduce the usage of traditional insecticides. We anticipate that one or more of the GRAS compounds that we identify in these studies will find immediate application in ATSBs, as they should be deployable without requiring additional regulatory approval. We also recognize the potential effects of these compounds on non-target species and plan to test them in the coming year on other fly species (see below).

List Objectives and specifically describe progress tied to objectives and deliverables in the approved proposal.

- **Develop odor blends that attract *Ae. aegypti* & *Ae. albopictus* in the context of sugar feeding.**
Work on this objective has begun, but results are still too preliminary to report.
- **Identify GRAS compounds that reduce mosquito lifespan, especially at sublethal concentrations.**
Our studies of the effects of diols on *Ae. aegypti* have gained momentum this summer with the addition of two team members to the project. We have made a number of important observations regarding diols and adult survivorship. These include dosage effects, sub-lethal effects, sex-dependency, and an apparent general lethality of diols in the context of sugar feeding (see supporting data below).
- **Conduct field trials of ATSB attraction in McLennan County (Waco) and Harris County (Houston), Texas**
These studies are scheduled to begin in the summer of 2022, as long as COVID restrictions do not impede our ability to work with Harris County Public Health.

Progress Assessment:

Overall our progress has moved forward at an increasing pace. Henry Lyons, an undergraduate assistant, initiated the sugar feeding assays from January through May as part of an independent study laboratory course. In June, Heidi Lindsley, a third-year graduate student, and Melissa Noreña, a part-time undergraduate assistant, both began working on this project. Despite the slow start, we have made significant progress in the first year. We have identified a number of GRAS compounds that reduce mosquito survivorship at sublethal concentrations (Objective 2). We anticipate of this proposal and expect to initiate trials related to objective 1 in the Fall semester. The second factor that affected our progress was the slow return to full access to research facilities due to COVID protocols. As part of our

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social distancing efforts, we limited the number of personnel who could be in the lab at any one time and restricted undergraduates to 1-2 days per week. As the Spring semester progressed and personnel became fully vaccinated, we gradually increased access to our laboratory. Following Baylor University guidelines, we have now been able to open the lab to full-time effort for graduate and undergraduate students.

List Milestones and assign a color. Be honest and critical of your work and solutions for overcoming challenges. If not completed list % completed.

*Months 1-12:

1. Test unitary odors, essential oils, and synthetic odor blends in olfactometer and dual choice assays.
2. Test 20-30 GRAS compounds for lethality by adding to sugar bottles and recording longevity.

Milestone 1 will be delayed by 1-6 months. Our olfactometer and dual choice assays began in the Fall semester and will continue through the Spring semester of 2022. We have essentially completed Milestone 2 on time, although we anticipate conducting more trials with varying concentrations (see below), through early 2022.

*Months 6-18:

1. Combine sugar feeding with attractive odors in olfactometer assays to assess upwind foraging behavior of female and female *Aedes* spp.
2. Follow-up testing with the most promising GRAS compounds to examine effects of single-feeding (24-hour) versus continuous feeding delivery paradigms on adult longevity.

Milestone 1 will be delayed until the start of the Spring semester and will continue until the end of year 2 (Months 13-24). Milestone 2 is in progress and we will move these trials forward for the other GRAS compounds following successful completion of Milestone 2 above.

Months 19-24:

1. Field evaluations of ATSB attractants in collaboration with in Harris County (Texas) Public Health, across the summer/fall seasons in selected sites.
2. Assess proportion of insects feeding on ATSBs and effects on local mosquito populations.
We anticipate starting the field trials in collaboration with Harris County Public Health, Vector Control, in the summer/fall of 2022.

*periods for which funding is requested

Plans for the following year:

In the coming year we plan to expand our testing of the GRAS compounds identified in Objective 2, specifically for lethality (continuous feeding) and sub-lethality (24-hour feeding) against *Ae. aegypti* and *Ae. albopictus* at various concentrations. We believe that these studies will be important for their use in modified ATSBs to produce the desired life-shortening in *Aedes* mosquitoes, while also ameliorating potential negative effects on non-target insects. We expect to produce a final ranked list of compounds that demonstrate the highest efficacy, especially in our 24-hour feeding paradigm. A short list of 2-3 of the best compounds will be moved forward for field testing. We also plan to test the most promising compounds against

the non-target species *Drosophila melanogaster* (vinegar flies) and *Musca domestica* (houseflies), which will serve as models for their effects on smaller and larger insects, respectively.

We have recently initiated odor attraction assays, with our initial studies focused on essential plant oils. We anticipate moving progressively into unitary compounds and synthetic odor blends. These assays will serve as the basis for odor-baiting ATSBs in field trials. Ultimately, we will combine attractive odors with GRAS compounds in dual choice feeding assays and compare with non-odor baited and sugar-only controls to ensure that mosquitoes are not only attracted to bait stations, but are not repulsed by the compounds in the sugar source. In 2022 we will also build prototypes of ATSBs, based upon the design that we submitted in our original proposal, which was derived from previous studies (Obenauer *et al.* 2015, Cohnstaedt *et al.* 2012).

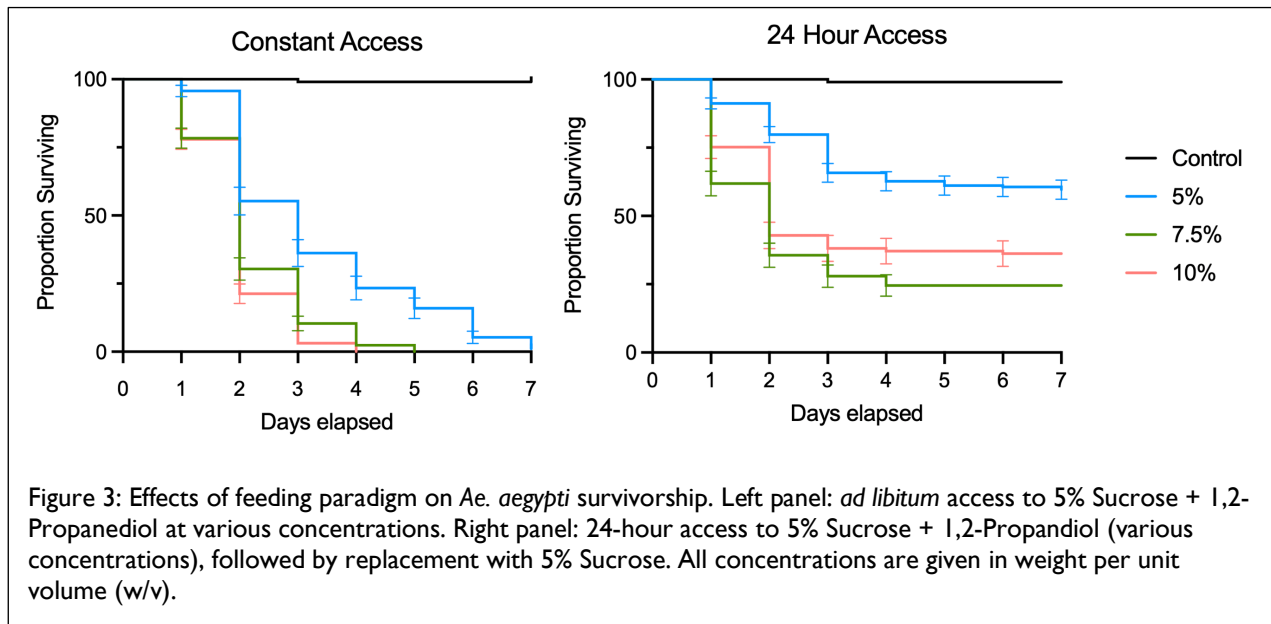
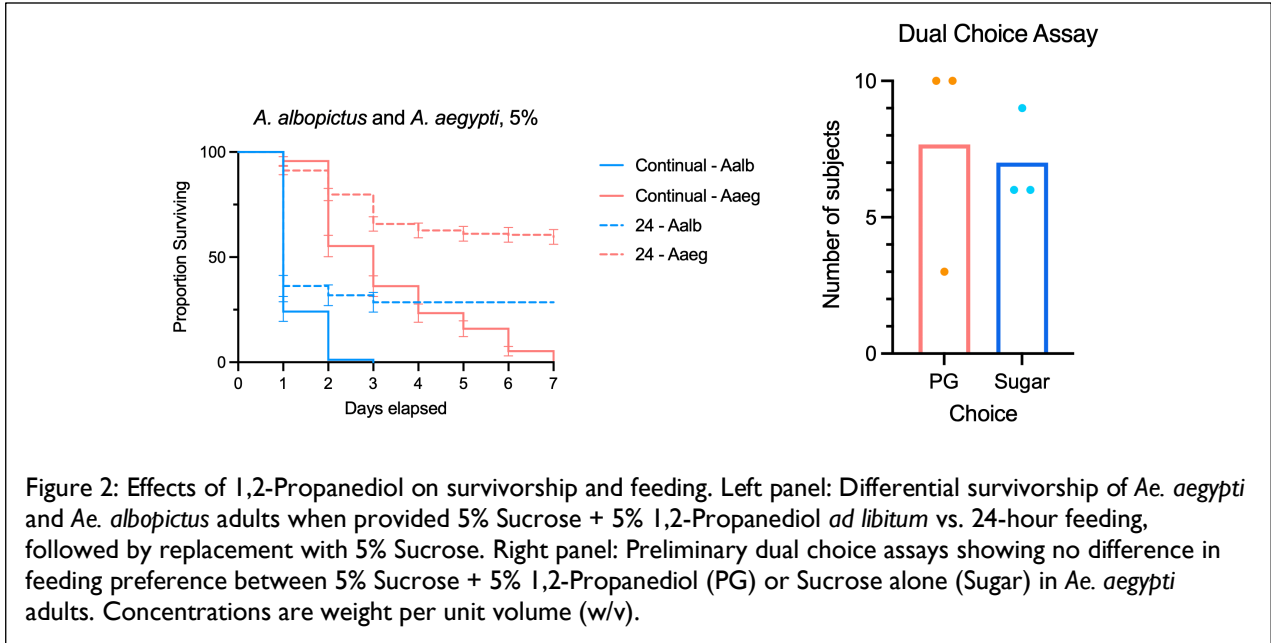
Conclusion:

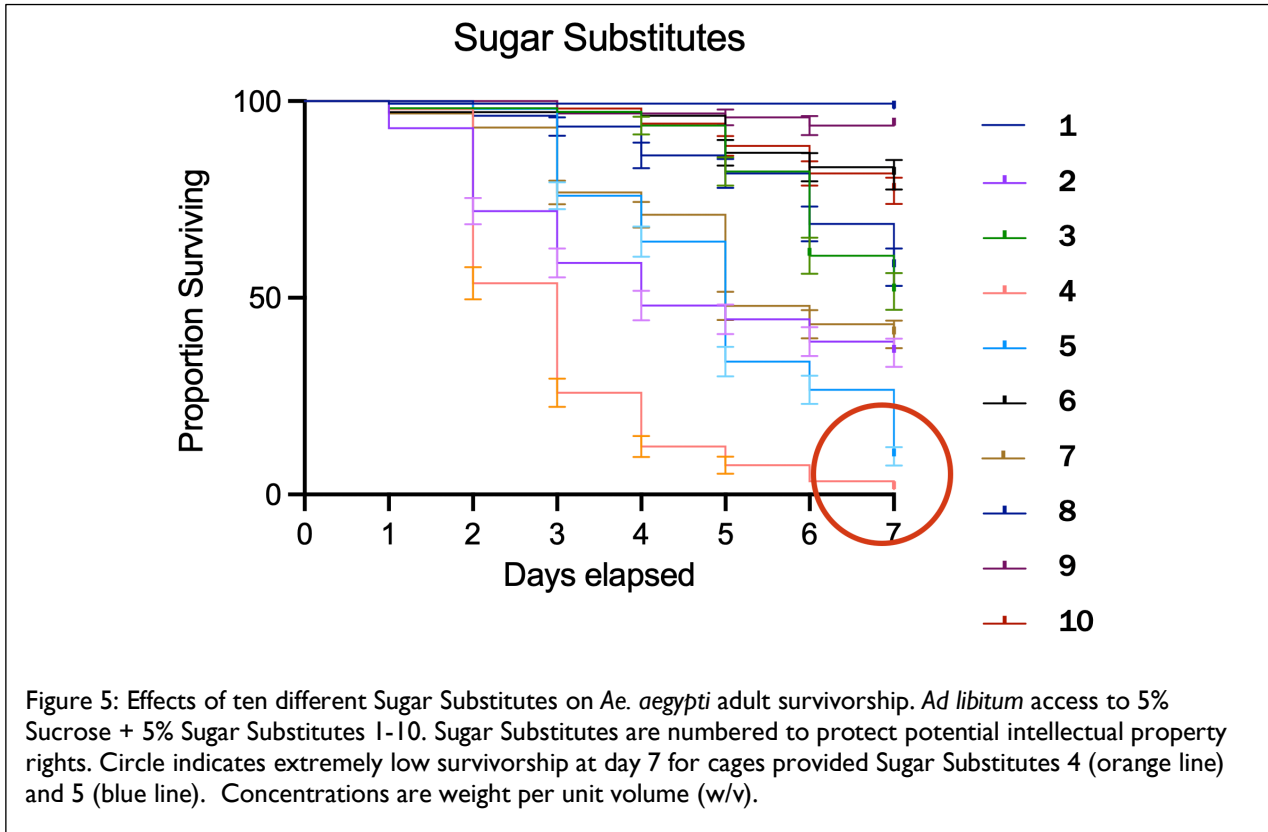
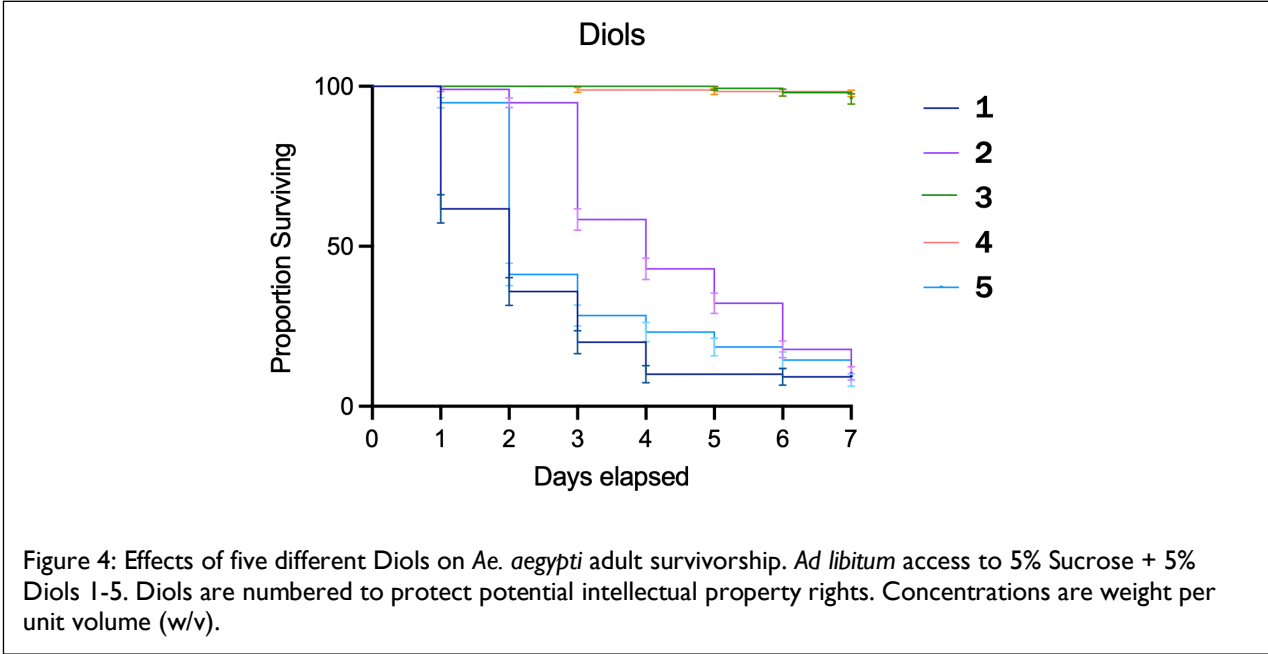
Despite a delayed start, we are pleased with the progress that we have made in 2021 toward identifying promising lead compounds for ATSB deployment. We anticipate prioritizing our list of GRAS compounds, leading to additional laboratory and field testing. Attractive odors are an important component of ATSBs and the overall goals of this project. We expect to identify odors that can be combined with GRAS compounds to attract both *Ae. aegypti* and *Ae. albopictus*. Importantly, we have started screening compounds in a sugar-feeding paradigm with both species. Moreover, we will use two fly species as models for measuring the effects of our most promising lead compounds in non-target species. These efforts are also a significant component of our overall goal of producing ATSBs that are as specific as possible at targeting vector mosquitoes in localized environments, while avoiding harmful effects on beneficial insects. We are especially excited about the prospect of utilizing non-traditional insecticides. Our expectation is that by using sub-lethal, life shortening concentrations of GRAS compounds, modified ATSBs may also help reverse target-site, metabolic, or behavioral resistance mechanisms in mosquito populations where they may have already developed.

Supporting Data:

1,2-Propanediol	Aspartame	D(+)Trehalose
1,6-Hexanediol	Acesulfame Potassium (Ace-K)	D-Glucose
1,3-Propanediol	Stevia	D(-)Arabinose
1,5-Pentanediol	Sodium Saccharin	D(+)Cellobiose
2-Methyl-1,3-Propanediol	Erythritol	D(+)Mannose
DL-Threitol	D(-)Fructose	D(+)Raffinose
Allulose	Beta-D-Lactose	D-Mannitol
Monk Fruit Extract	D(-)Ribose	D-Xylose
Neotame	D(-)Galactose	Sorbitol
Xylitol	D(+)Maltose	Sucrose

Figure 1: GRAS compounds utilized in feeding assays.





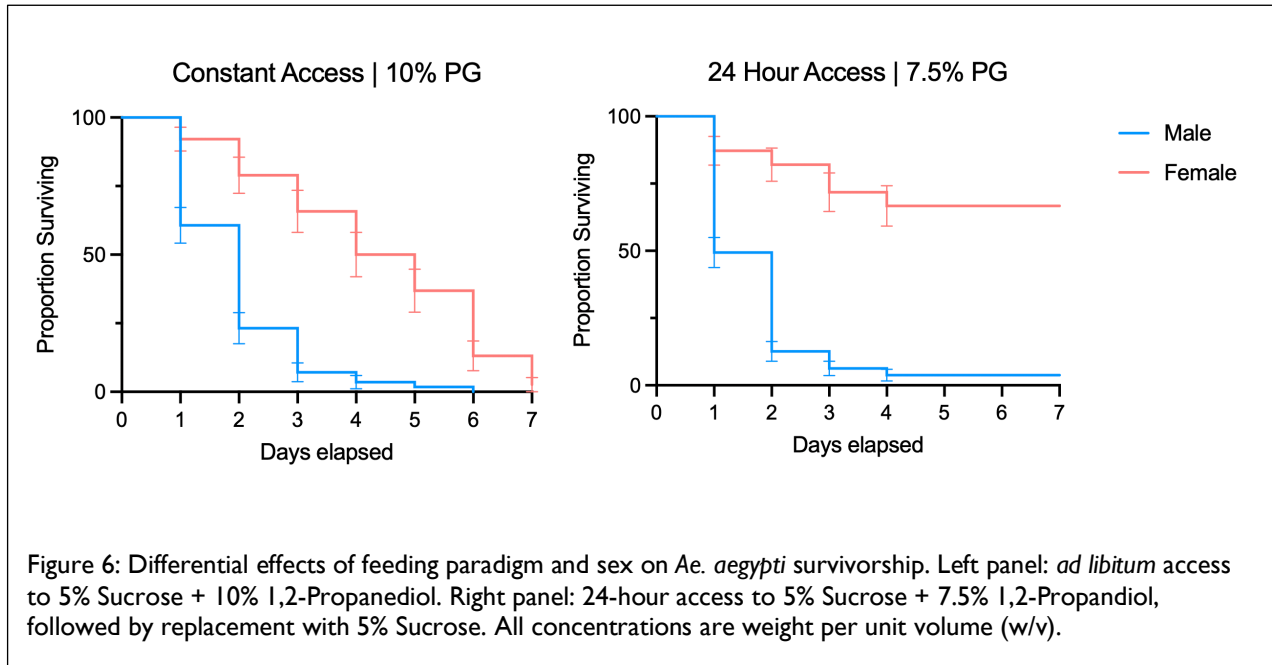


Figure 6: Differential effects of feeding paradigm and sex on *Ae. aegypti* survivorship. Left panel: *ad libitum* access to 5% Sucrose + 10% 1,2-Propanediol. Right panel: 24-hour access to 5% Sucrose + 7.5% 1,2-Propanediol, followed by replacement with 5% Sucrose. All concentrations are weight per unit volume (w/v).