AMCARF Project Status Report

This report will be used to communicate the progress, successes, and challenges of your AMCARF Funded project. The report will be used by the Review Committee to assess overall productivity of the project and its continued alignment with the AMCARF objectives. It will also be used to make future prioritizations of research and development efforts.

The report should not exceed 8 pages of text. Please include any additional attachments with key figures that may highlight critical findings. Use the following headings to build your report:

Individual filling out this form: Brian Byrd, PhD, MSPH

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Report Type:

	Report Type	**Report Deadline**
	Progress Report	
✓	**Annual Report**	Monday, Dec. 16th 2019

Project Title: Rapid identification and characterization techniques for mosquitoes of public health importance

AMCARF project number: 2019-01

Project Cost: $29,609.14

Project Leader: Brian D. Byrd, PhD, MSPH

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

-Scott Huffman, PhD (10% Annual Effort)

-Stephanie Richards, PhD (Cooperating Laboratory)

-Multiple mosquito control districts (Contributors)

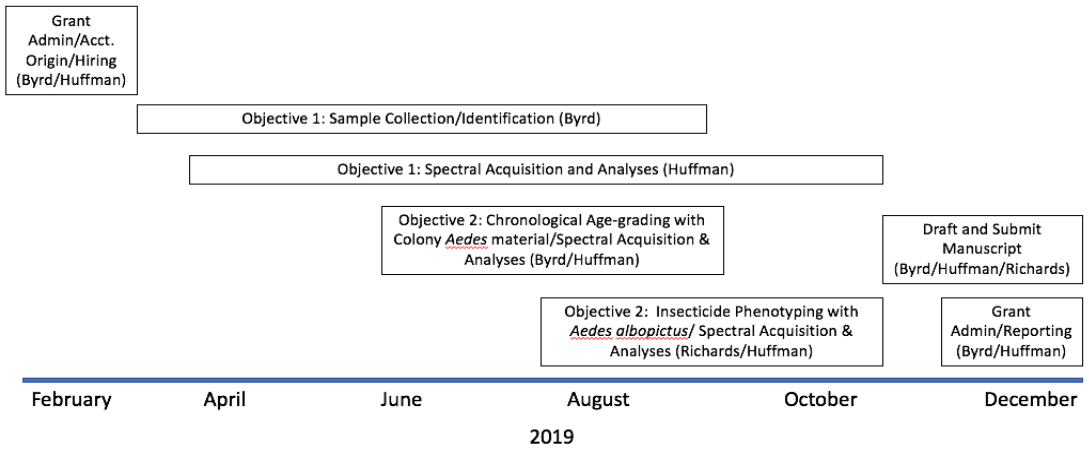
Project Objectives: (Bullets as stated in the approved proposal. Add new or modified objectives.)

Objective 1. Increase the scope and number of mosquito species in the MIRS assay by expanding the spectral library

Objective 2. Provide "proof of principle" evidence for the use of MIRS to age-grade and phenotype insecticide susceptibility

SUMMARY OF TOTAL PROJECT PROGRESS: We have made major progress on both principle objective and have generated usable data that will be used to generate two additional manuscripts. One manuscript will describe the use of MIRS for age-grading mosquitoes, an additional manuscript will be drafted to describe the use of MIRS for rapid container *Aedes* eggs. We submitted one manuscript (*Applied Spectroscopy*) in August and this manuscript was reviewed and returned for revisions. We are addressing the reviewer comments. Most reviewer comments were minor or represented a need for us to clarify some points or methods. We fully expect this manuscript to be accepted forthwith.

Project Timeline



Key Research Accomplishments: (Bulleted list of accomplishments from this project)

Objective 1 Accomplishments: Increase the scope and number of mosquito species in the MIRS assay by expanding the spectral library

- We have measured over 1000 IR spectra from mosquitoes. Most with tibia measured + subset external abdomen + subset abdominal "squash". Some spectra were obtained from container-*Aedes* eggs.
- We have collected, obtained, and reared over 1,000 mosquitoes (See below table for status)
- We developed a systematic sample storage/archival system for post-MIR evaluation (e.g., DNA or repeat/blinded morphological identification to verify any "failed" infrared classifications.
- We refined our measurement techniques with three improvements: aperture optimization, water vapor control, substrate selection
- 3 technicians (graduate and undergraduate students) were trained on MIR spectral acquisition (supported by AMCARF and other funding)
- Field collections were overall successful. Collections in SC, NC, FL, CO, UT, and TN have been completed and yielded specimens. Additional field collected specimens were sent from LA and FL.
- We have demonstrated a "proof of principle" trial of using MIR for species identification of container *Aedes* eggs.

IMAGE REDACTED FOR WEB (PUBLIC) VERSION
Figure: Simple principal components analysis (PCA) demonstrating separation of *Ae triseriatus* (blue) and *Ae. albopictus* (red) eggs. We fully expect clearer separation using PLS-DA or other analyses as we've used for our species identification. We have added 3 additional container-*Aedes* to this egg assay. We expect to submit a manuscript from this work in 2020 to the *Journal of the American Mosquito Control Association*.

MIR Spectra Acquisition Status (Dec 15th, 2019)

Species	MIR Spectra Obtained (Status)	Collection Type
Ae. albopictus	Yes	Field & Colony Material
Ae. aegypti	Yes	Field & Colony Material
Ae. triseriatus	Yes	Field & Colony Material
Ae. japonicus	Yes	Field material + F1 eggs
Ae. hendersoni	**No**	**For 2020**
Ae. atropalpus	Yes	Field & Colony Material
Ae. epactius	Yes	Field & Colony Material
Ae. sierrensis	Yes	Colony Material
Ae. notoscriptus	**No**	**For 2020**
Culex pipiens	No	In Progress*
Culex restuans	Yes	Field Material
Culex tarsalis	Yes	Colony Material
Culex quinquefasciatus	Yes	Field & Colony Material

*We have to address the *Cx. pipiens/quinquefasciatus* hybrids found in most of NC; need additional Cx. pipiens from further north (2020)

Additional spectra obtained include: *Cx. salinarius* (Field Collected)
Species collected, initially processed : *Cx. nigripalpus, Cq. perturbans, Cs. melaura, Cx. pilosus, Cx. erraticus*

Objective 2 Accomplishments: Provide "proof of principle" evidence for the use of MIRS to age-grade and phenotype insecticide susceptibility

- We generated MIRS spectra to evaluate chlorpyrifos susceptibility, presence and concentration in/on *Aedes albopictus* at 3 doses (30 micrograms/mL, 45 micrograms/mL, and 100 micrograms/mL) for 45 minutes in a standard CDC bottle arena (provided by Dr. Stephanie Richards, ECU). We generated MIRS spectra to also evaluate bifenthrin susceptibility, presence and concentration in/on *Aedes albopictus* at a single dose (11.8 micrograms/mL) for 15 minutes in a standard CDC bottle arena (provided by Dr. Stephanie Richards, ECU. Infrared spectra have been measured on the tibia of 57 *Aedes albopictus* that were phenotyped as susceptible or resistant to either chlorpyrifos or bifenthrin. An additional 14 tibias from a control set (not exposed to either insecticide) were measured also. There are clear differences between the infrared spectra of the exposed and control mosquitoes. There are some spectroscopic differences between the susceptible and resistant mosquitoes. However, we currently believe these differences are a methodological artifact (likely base on the bottle assay application) and are not necessarily biochemical (genotype or phenotype) differences between the mosquito classes. Further study is required.

- We have 3 experimental trials with age-grading to report at this time. We successfully classified *Culex quinquefasciatus* mosquitoes categorically (e.g., old [>2 weeks old] versus young [< 1 week

old]); these mosquitoes were not taken in small age cohorts. However, even with this "rough" categorical application, we were able to discriminate between the age cohorts. This is important, as older mosquitoes, in the absence of transovarial pathogen transmission, are far more important epidemiologically.

IMAGE REDACTED FOR WEB (PUBLIC) VERSION

Figure: Simple principal components analysis (PCA) demonstrating separation of <1-week-old (red) and >2-week-old (blue) *Culex quinquefasciatus*. We fully expect clearer separation using PLS-DA or other analyses as we've used for our species identification.

- We have also completed a time course rearing experiment with *Culex tarsalis* (cohorts of 5-35 days of age) to determine if MIRS can detect chronologic age differences; spectra have been acquired from many of these samples and the age bin analysis will be completed shortly.
- We have attempted two rounds of age grading analyses of *Aedes atropalpus* with a graduate student and we have run into some challenges obtaining the necessary cohorts of mosquitoes experimentally. We had some contamination (? IGR) in the laboratory that knocked down a couple of colonies in Sept./October 2019. We have recovered from this and currently have an additional cohort experiment underway with *Ae. atropalpus*. We may have to repeat the collection of these data in 2020. However, these data for the student's thesis and we are confident it will be completed. He is expected to present some of these data at AMCA along with his *Cx. quinquefasciatus and Cx. tarsalis* data. We are also planning on age-grading experiments with *Ae. albopictus*. Our *Ae. epactius* colony materials (MX/CDC and Field Collected TX -2019) were lost to the suspected contamination. We will not be completing these analyses until late summer 2020 at the earliest.

Reportable Outcomes:

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

- We submitted a manuscript (*Accurate Classification of Mosquitoes with Infrared Spectroscopy and Partial Least Squares-Discriminant Analysis*) for submission to *Applied Spectroscopy*. The manuscript was peer reviewed and returned for revisions. Most of the reviewer comments were minor and have been addressed. We are adding some additional figures to address some conceptual gaps the reviewers experienced. We expect this manuscript to be accepted before the 2020 AMCA meeting.

  --UPDATE 1/15/2020: The manuscript has been accepted for publication --
- We have received a NC Biotechnology Center "Biotechnology Innovation Grant" to partner with Leading Edge Associates (LEA) in order to determine the commercial viability of the work funded by the AMCARF. LEA will conduct a market analysis to inform if and how the technology may be used for mosquito control.

- We submitted three abstracts to the 2020 AMCA meeting (1 oral, 2 posters) that describe some of the work supported through the AMCARF. See attached.

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

- No major advancement to describe at this point. However, the use of spectroscopic, image, and sound analyses for identification of mosquitoes is growing. We fully expect that in the future, as this technology is automated and becomes more cost effective, these technologies, including IR analyses, will become more commonplace.

Progress Assessment:

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

Milestones:

Sample Collection/Identification: See above table. We have met most of the goals for this project. We experienced a drought in the areas of WNC where we normally collect *Ae. hendersoni*. This will have to be addressed in 2020. We are still waiting on obtaining *Ae. notoscriptus* samples from colleagues.

Spectral Acquisition: We have collected meaningful spectral data in the past 10-11 months supported by the AMCARF that will result in peer reviewed manuscripts. We have specimens still needing spectral acquisition and we have technicians in place to continue this work through the summer of 2020.

Chronological Age Grading: See above re: suspected IGR contamination. We have promising preliminary data with *Cx. quinquefasciatus* and *Cx. tarsalis*. Additional spectra are currently being obtained on *Ae. atropalpus.* We expect to obtain spectra on either *Ae. albopictus* or *Ae. aegypti* in early 2020.

Insecticide Phenotyping: See above. We have reduced the priority of this aim given the first round of data that suggest that the insecticide or assay itself is separating the species. We are fundamentally interested in teasing this out in the future. However, given our other promising data (eggs, age, species), we are going to focus on getting the manuscripts out for the aims that are presently working.

Draft and Submit manuscript: Ahead of schedule, we drafted for submission a manuscript about the technical spectroscopic approaches for submission in *Applied Spectroscopy*. Subsequent papers (application work supported here) will cite these methods. We expect to submit two manuscript to the *Journal of the American Mosquito Control Association* (*JAMCA*) in 2020 based on the attached AMCA Annual Meeting abstracts (attached).

Plans for the following year: We expect the *Applied Spectroscopy* manuscript to be published in 2020; the AMCARF is acknowledged in this publication. We also plan to complete the analyses for the container *Aedes* eggs manuscript and submit to *JAMCA*. The age-grading work will be published as a thesis in 2020 and we also expect to submit a manuscript with these results to *JAMCA*. We will continue to add additional mosquito species to our internal spectral library and will add *Ae. hendersoni* as soon as phenologically possible. We still hope to obtain the *Ae. notoscriptus* from our CA colleagues. Future work will include development of automation methods for identification. We have internally discussed

seeking Federal funding for bringing this technology/research to scale with a larger mosquito library and combined age analyses.

Conclusion: This project demonstrates the use of mid-infrared spectroscopy for phenotyping (species identification & physiological status) mosquitoes of public health importance. The rapid identification of container *Aedes* eggs may be an operational advancement of interest to all conducting container *Aedes* surveillance. We envision labs shipping ovistrips to regional labs that can rapidly (same day) provide reports without the need for consumable PCR reagents. A larger robust spectral library of mosquitoes is currently being developed.

Supporting Data:

Manuscript Images:

IMAGES REDACTED FOR WEB (PUBLIC) VERSION

Images submitted in final report were submitted for publication to the journal Applied Spectroscopy. This publication has been accepted, images are expected to be publicly available online in Feb. 2020

https://journals.sagepub.com/home/aspc