Final Progress Report for FCCMC Research

The Florida Department of Agriculture and Consumer Services (DACS)

(Contract 23586)

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Title: “Development of a robust olfactometer for testing mosquito control agents”

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Abstract:

The glass and metal olfactometer/wind tunnel has been built as planned to incorporate ozone, and also modified to incorporate the UV lights needed for full decontamination by persistent pesticides and repellents. This will now allow testing of persistent mosquito repellents and insecticides such as transfluthrin that often leave biologically active residues for long periods of time. Testing of this new device has shown that residual (“contamination”) transfluthrin kills mosquitoes for more than 10 days after initial exposure. Cleaning protocols using live mosquitoes have been carried out to a limited extent and show that the decontamination process is able to remove the contamination (limit mosquito death) and should allow routine testing of subsequent hard to remove agents.

Introduction: (modified from our proposal)

This proposal was to design, build and test an olfactometer for evaluating mosquito control chemicals which would incorporate features for improved performance. Most current devices use polymeric materials such as Plexiglas in part of the construction, and those parts can absorb chemicals into their structure as well as outgas chemicals such as residual monomers which could also alter mosquito behavior or survival. These accidental releases of volatile species can complicate interpretation results such as degree of repellency. We proposed to incorporate a fully metal and glass design for all of the key parts (eliminating plastics), and to add an ozone cleaning system as an integral part of the usage. This would prevent long delays in cleaning the olfactometer between runs, and provide a cleaner air stream for testing. In addition, it was planned to allow the testing of hard to remove pesticides and repellents such as the pyrethroids, which are sometimes avoided in olfactometer usage due to their resistant residues on and in surfaces, and their extremely low concentrations...
needed for effectiveness. Some olfactometers provide a new, clean environment by simply disposing of the plastic parts between runs.

The olfactometer was designed and built in conjunction with a local company with experience in the area and some testing has been carried out using *Aedes aegypti* mosquitoes supplied by the USDA in Gainesville, Florida. The design will be made available to other investigators, and we anticipate that this will allow more convenient and reproducible testing of the most difficult control agents available now and in the future.

Preliminary testing in glass jars showed that ozone was insufficient to decontaminate the surfaces, and UV-light was needed to speed degradation. This possibility was anticipated in the original proposal. Four 85 Watt UV bulbs were added to the unit already built and that seems to provide sufficient power to degrade the residual organic materials present.

**Methodology:**

I. **Build glass and metal wind tunnel with UV and/or ozone cleaning capabilities**

This part of the project was contracted to Sigma Scientific LLC of Micanopy, FL. Sigma had finished all major construction on the wind tunnel and air handling system which incorporated an ozone generator. Construction took longer than expected because of some supply issues and design modifications which were incorporated as construction progressed. For instance, we eliminated some of the glass blowing features which would have resulted in a much longer delay. Based on the glass jar results, it was clear that UV light sources should be added. We met with the Sigma group several times and discussed how to incorporate relatively low cost tanning UV lights into the wind tunnel. This design was implemented, and on May 16, 2017 the UV light array and protective quartz tubes which are part of this second
stage modification of the original design were finished and installed. We originally expected to have a functional wind tunnel where we could run initial tests and fine tune the testing method for maximum comparability to other studies by the middle of March 2017. However, there were delays with the more complex installation and safety features that were essential to incorporate. However, the work was finished and the planned testing was carried out in the last quarter.

Because of the large size of the unit, and some uncertainty about where to best place it, we have decided to leave it at Sigma for initial testing. Sigma had offered to allow us to do this initial work at their site which we gratefully accepted. We have a very good working relationship with this group, and plan to leave the wind tunnel there for the foreseeable future. In the meantime, we are exploring where it might best find a more permanent home, but want to have the modifications and improvements incorporated at this more accessible site and to have a significant amount of testing done at that site as well. Despite delays, we were able to have a fully functional device, tuned and tested by the scheduled end of the project. Two photographs of this second-stage design completed tunnel are shown in figures below with the UV lights on. The quartz tube housing allows the UV light to expose the interior while the soda-lime glass used in the rest of the construction blocks about half of the UV emitted. This amount of UV is still potentially damaging to the eyes of workers nearby, so precautions are being added to prevent accidental exposure. These include warning signs, an opaque blanket available to cover the olfactometer and a timer for overnight use.
As mentioned in the third quarter report, we have shown that contamination resulting from testing transfluthrin resulted in killing mosquitoes for at least 10 days with just air flow occurring. When the UV lights were added, a 15 minute exposure decreased the rate of killing, but a longer time (about one hour) was needed to essentially eliminate early killing of the c. 200 mosquitoes placed in the device. This type of testing has been carried out twice, and showed the need for clear protocols for contamination and cleaning. One trial resulted in a likely contamination of a filter unit that was not subsequently exposed to the UV light. This testing is continuing with funding from a variety of other sources. The other testing included about 250 mosquitoes, and only 5 had died overnight. This was a normal loss, i.e., no effect of transfluthrin.

II. Additional add-ons.

Also keeping within budget for the second-stage add-ons, we have modified some of
the inlet ports and the insect introduction port.

**Acknowledgements:** We are grateful for the guidance and collaboration in constructing, testing and modifying the novel designs by Dr. Sandra Allen of the USDA, as well as Jim Estaver and Rudy Strohshein at Sigma Scientific Laboratories who are providing space in their facility to carry out the next round of tests with live mosquitoes.

**Results:** We have finished the basic construction of this novel device for testing mosquito behavior when exposed to a wide variety of chemicals which are difficult to test with current devices, and are looking forward to using it for a variety of repellents and pesticides.