Novel Lethal Ovitrap and Larvicidal Chips for Control of Aedes Aegypti and Aedes Albopictus

Hi, my name is Casey Parker; and I'm a master's student at the University of Florida, working with the Lethal Ovitrap for control of container-breeding mosquitoes.

And I'm Kristen Stevens. I'm also a master student here at the University of Florida, and I'm working with a larval chip to control container-breeding mosquitoes.

Today both of us are going to talk about our respective research.

I'm sure that we're all aware of the threat that mosquitoes pose to the human population. They're a major disease transmitter and are responsible for approximately 725,000 deaths per year due to diseases like malaria. And really, 725,000 is considered to be a very conservative estimate.

In addition to causing all of those deaths annually, mosquitoes infect hundreds of millions of people with various diseases and afflictions every year. As I just mentioned, mosquitoes are responsible for infecting humans and animals with a variety of diseases and arboviruses. Mosquitoes are also found on every continent of the globe excluding Antarctica. They are excellent at transmitting diseases, and malaria is probably the most well-known. There are 10 new cases of malaria every second, and 300 million to 500 million infected people every year.

We also spend a great deal of money in attempts to control mosquitoes, approximately $150 million a year. And pictured here at the bottom of the screen is plasmodium vivax and anopheles gambiae, which is the malaria and the mosquito vector.

But Kristen and I don't work with malaria or the anopheles mosquitoes. We work with Aedes aegypti and Aedes albopictus and novel methods for their control. These species have been in the news recently, due to outbreaks of zika as well as dengue and chikungunya. The most recent of these outbreaks is the zika virus, which has not shown local transmission in the U.S.A. yet. But as of March 16, 2016, there have been 258 travel-associated cases in the U.S.; 18 infections in pregnant women; and 6 sexually transmitted cases. It's hard to predict how and where the zika virus will continue to spread, but it is expected to continue to spread.

Aedes aegypti is one of the vectors of zika, and it has a very unique feeding behavior. They prefer to feed primarily during the day but will also feed at night. They're highly adapted to develop in artificial containers around the home and will bite indoors and outdoors. They're found throughout the world and are also the primary vectors of dengue, chikungunya and the zika virus in the Americas.

They're easily identifiable by the distinctive lyre shape on the thorax, which you can see in the bottom picture on the screen.

These maps give an approximate distribution of Aedes aegypti according to the CDC as of 2015. As you can see, they are widely distributed throughout the world and established in the U.S. Their range tends to stay in the more southern regions of the U.S., and most of that is in the Southeastern Region.

Aedes albopictus is one of the most widespread mosquito species and is considered highly invasive. It demonstrates the same unique daytime feeding behavior as Aedes aegypti, but is a much more aggressive biter than aegypti. These mosquitoes are also highly adapted to container breeding, and will bite both indoor and outdoor settings. They can be found globally, just like Aedes aegypti.

The zika virus was isolated from Aedes albopictus in Gabon in 2007 outbreaks, so it is expected to be a possible threat in zika virus transmission but has not yet been confirmed.

Aedes albopictus has a very similar distribution to Aedes aegypti; however, their range is not constricted to just the Southeastern U.S. Their range expands much farther northward in the U.S., and these maps were produced by the CDC. The map displays data that was available at the time, but these populations are consistently changing. Therefore, these mosquitoes may be in places that are not necessarily shaded on the map and not consistently present in places that they are shaded.

As I mentioned that Aedes aegypti and Aedes albopictus are container breeders, and I would like to expand on that just a little bit.

Aedes aegypti and Aedes albopictus are capable of developing in small containers. Natural containers would include things such as leaf axles or tree holes, but these mosquito species are very good at exploiting the numerous artificial containers that are found around a home.

As you can see from the picture on the slide, there are a wide number of places that the mosquitoes can develop. Larvae are able to develop in containers as small as a bottle cap from a soda. Their exploitation of these artificial containers is likely how these mosquitoes were brought into the U.S. Tires and lucky bamboo plants make excellent mediums for moving these mosquito species overseas. Eggs or larvae were likely present in the containers and, upon their arrival in the U.S., completed their development and began to establish.

Aedes aegypti have a very anthropophilic nature and will feed on humans readily. Not surprisingly, the larvae tend to occupy artificial containers around a home. These can be flooded wheelbarrows, flower pots, or the saucers beneath them, clogged rain gutters, bird baths, tires, or even litter on the side of the road.

These species are highly invasive and difficult to control due to their unique feeding behavior and their exploitation of natural and artificial containers. Their human blood feeding preference makes them a species of concern for disease transmission, especially since their distribution seems to continue to expand.

Kristen and I are working on two novel methods of controlling these mosquitoes. When used together, these control methods may be capable of reducing Aedes aegypti and Aedes albopictus populations.

So to start off with my area of research, I would first like to introduce the lethal ovitrap. These have been used in recent years to try to control container-breeding mosquitoes like Aedes aegypti and Aedes albopictus. They're essentially a container with water and a pesticide-treated strip or some other lethal agent. There have been many permutations to this, including different colors and patterns, different insecticides, and even biodegradable lethal ovitraps.

The major problems that we have identified with this first off are that their open design allows for both a lot of air movement inside the trap and a high rate of rainfall collection. The pesticide-treated strips also have a low residual activity, which will eventually become ineffective at controlling the mosquitoes. In addition, when the insecticidal activity is completely gone, the container can actually become an additional breeding site for mosquitoes.

These containers are also not competitive with other containers that are present in nature, such as tires and birdbaths.

So we came up with this idea of a novel lethal ovitrap which would eliminate the shortcomings of the standard lethal ovitrap. This trap is pictured here, and we call it the durable dual action lethal ovitrap or DDALO for short.

This trap is made out of a very durable plastic that resists weathering and degradation. Additionally, the interior of the trap is treated with a formulation that includes slow release polymer, a larvicide, and an adulticide. The larvicide is an insect growth regulator, more specifically a juvenile hormone mimic, that causes a disruption in the life cycle of the mosquito and prevents successful emergence. This larvicide has low mammalian toxicity and is actually allowable in drinking water for mosquito control at a rate of 0.01 milligram per liter in some places around the world.

In addition, the trap has a textured interior surface to encourage oviposition and a narrow entrance that does not allow for too much water buildup and also provides palm shade and an area safe from wind for the mosquitoes to oviposit. This trap is currently undergoing EPA registration and testing.

My research objectives for this trap were first to evaluate its efficacy and the effects of aging on the trap in an indoor and outdoor environment; determine if this trap would be a competitive container with other containers in the environment; determine the effect of the DDALO on a small cage population of mosquitoes; and the preparation for field studies, which included larval habitat surveillance as well as adult surveillance.

To evaluate the trap efficacy and the effects of aging, I placed a treated or an untreated DDALO inside of a cage. Twenty recently blood-fed mosquitoes were then released into the cage and provided with sugar water. They were allowed to oviposit for a period of five days; and at the end of the five days, the adult mortality was reported, and the traps were removed from the test cages. DDALOs were flooded with water to induce egg hatch, and the resulting larvae were reared and the pupation was recorded.

These methods were repeated every month with traps that were aged either in an indoor or outdoor location, and this was repeated for a period of six months.

Here we can see the adult mortality associated with the DDALOs over time. The *x* axis shows the age of the DDALO in months, and the *y* axis shows the percent adult mortality. This graph is split into four sections, based on whether or not the trap was treated and the location of where the trap was aged.

As you can see, there was not a substantial difference in adult mortality between the indoor and outdoor aging locations; but we did see that the adult mortality was high in the first two to three months and gradually declined to approximately 50% by the sixth month.

This graph is set up the same as the last graph, except the *y* axis is now the number of number of larvae that successfully pupated. What is interesting here is that throughout the duration of the six months of aging, the treated traps did not allow for any mosquitoes to successfully emerge. Therefore, we were able to maintain a 100% immature control with this trap for a minimum of six months. However, the adult mortality was consistently decreasing while the immature mortality was not.

The next thing I wanted to evaluate was how this trap measured up to other containers that might be present in the environment. For this experiment, I released 20 recently blood-fed mosquitoes into a cage that included a resting site and all of the containers pictured at the bottom of the screen. These are a plant saucer, a Mason jar, inverted cups, and either a treated or untreated DDALO.

The mosquitoes were allowed to oviposit for a period of five days; and at the end of the five days, all of the containers were flooded, and the number of larvae in each container was counted and recorded to determine the oviposition preference.

The *x* axis on this graph shows the cage treatment, so cages with the treated DDALO were considered treated cages; and cages with an untreated DDALO were considered untreated cages. The *y* axis shows the number of larvae resulting from each container. And as you can see from the data, a vast majority of larvae that were counted resulted from the untreated DDALO. The most competitive site with the DDALO was the inverted cup apparatus, followed by the Mason jar and then the plant saucer. What is interesting here is that the treated DDALO did not even allow the eggs to hatch and produce larvae.

I also collected data on the number of eggs from each container; but this data was very similar and is actually not being presented today.

And the last lab study that I wanted to do was the multigenerational cage study on a small population of Aedes. This study was very similar to the last study as far as the setup goes. I released 20 recently blood-fed females into the age, and the mosquitoes were provided a resting site; sugar water; a plant saucer;

Mason jar; the inverted cup; the standard ovitrap, which is the new addition; and either a treated or an untreated DDALO.

The standard ovitrap was added so that weekly egg counts could be collected, and mosquitoes were blood fed daily; and the eggs on the tongue depressors were collected from the standard ovitrap once a week, counted, and replaced into their original cage. Larval diet was provided to all cages as needed; and at the end of the four-week period, all adult mosquitoes were aspirated to determine the final cage population.

On the left-hand side, we have a graph that displays the number of eggs collected from the standard ovitrap over the four-week aging period. And the red line is from the treated cage, and the purple line is from the untreated cage. After the first week, the number of eggs between the two cages did not significantly differ. After the first week, though, you can see that there were more eggs resulting from the untreated cages in comparison to the treated cages.

When we look at the number of live adults in the treated versus the untreated cage on the right-hand side of the screen, we can see that although we did not completely eliminate the cage population, there were approximately 400 fewer mosquitoes in the treated cage when compared to the untreated cage, which is a significant reduction.

I also did some preparation for field studies at a site in Thessaloniki, Greece. This consisted of monitoring larval development sites as well as adult surveillance. This campus has practically no vector control, and is an example of what naturally happens with a population that has no control. This campus has a history of high mosquito populations and also can be split into two distinct zones, residential and an agricultural zone.

For the larval surveillance, we split the campus into those two zones and thoroughly inspected the campus for active larval habitats. Active larval habitats were considered sites with water that had larvae present in them, and these habitats were categorized in one of seven ways. And the surveillance was done once every two weeks for three months. We also monitored the temperature and rain activity.

The top graph represents the larval development sites in the residential zone, and the bottom represents the larval development sites in the agricultural zone. The *x* axis shows the date the data was collected, and the *y* axis shows the number of each container type. The different colors on each graph correlate to a specific container type.

Although this graph may look very business, the take-home points are the primary larval habitats; and the two zones were similar in that they shared the water drainage system as a major larval habitat but different because while the residential zone had a great deal of barrels, buckets and flowerpots, the agricultural zone had a great deal of tires responsible for the larval activity. There were also two rain events that caused small fights for the number of larval habitats, which was expected due to the flooding of the eggs.

And we can use this data in future studies because this gives us an idea of the primary competitive containers in each site, so we know where to target our treatments when we do a treatment.

The last part of the surveillance study was monitoring the adult population; and to do this, I used three different trapping methods: the standard ovitrap, BG sentinel trap, and the CDC light traps.

Six sampling locations were chosen, three on the agricultural side and three on the residential side. These traps were put out once a week except for the standard ovitrap, which was constantly out. The tongue depressors in the standard ovitrap were collected and replaced with new ones once a week. The eggs from the tongue depressors were counted, hatched, reared to adulthood and then identified to species.

BG sentinel traps and the CDC light trap catches were brought to the lab, where they were frozen and identified as adults. This was done weekly from the end of May to the beginning of September to determine the adult population.

These three graphs show the mean number of mosquitoes caught with their standard errors. The top graph is the standard ovitrap, which is actually displaying the number of eggs collected from the tongue depressors. The middle is using the BG sentinel trap, and the bottom graph is using the CDC light trap.

The light blue represents catches from the residential area, and the dark blue represents collections from the agricultural area. There was not a significant difference between the two zones in the number of mosquitoes or eggs collected; but the first trap to detect aegis albopictus was the BG sentinel trap in the last week of May, followed by the CDC light trap and then the standard ovitrap. However, the standard ovitrap was an effective monitoring tool for aegis albopictus because it detected eggs throughout the season after the initial detection in the second week of June. However, it requires more time because it requires the rearing of the egg; but it doesn't require a batter pack like the CDC or the BG sentinel trap.

And while the CDC light traps were effective in detecting some aegis albopictus, the BG sentinel trap was better at detecting adult populations throughout the season, as you can see demonstrated by the higher number.

And this surveillance was used to compare to a treatment year in 2015. This data is currently being analyzed and put together for a publication that will be available, hopefully, in the coming month.

That concludes my research with the DDALO, a novel lethal ovitrap. I'll now hand it over to Kristen, who is discussing the sister project of the DDALO, larvicidal chips.

Hello, again.

I'm going to be talking to you about my research, which is on these larval chips. I (inaudible) Casey, and I'll show you the insect growth regulator. These are chemical substances that disrupt the action of insect hormones controlling molting, maturity from pupal stage to adult, and other growth functions.

There are a couple of classes of different IGRs, such as juvenile hormones, which (inaudible). Juvenile hormones are produced by insects; and when they're disrupted, the insect is unable to molt properly from its last larva stage to adult. And that can be seen here on this slide with this cockroach, for example. When going to molt to adulthood, his wings were not able to fully develop; so he has what they call twisted wing syndrome. In mosquitoes, it's a little different; but it still affects the way that they molt to their adult stage.

The IGR that I'm working with specifically is pyriproxyfen. Pyriproxyfen is very stable in the environment, so it lasts over a long period of time, and it is also labeled for mosquito control at 0.01 milligrams per liter allowable in drinking water; and it can be used as a surface application. Pyriproxyfen is a relatively safe chemical to use because it only affects insects because insects are the only ones that will produce these juvenile hormones. So it doesn't affect things like birds or mammals or other organisms that are residing in these mosquito-breeding habitats.

Here is my larval chip. Like I said, the active ingredient is pyriproxyfen. The pyriproxyfen is put into a polymer and pipetted onto small little tiles for my research. These tiles, the idea behind it is they can be placed into these mosquito-breeding habitats. They can be put in the planters, in the birdbath. Anywhere where these mosquitoes are breeding these little tiles can be thrown in and will treat the environment over time. They're non-toxic and they're long lasting. So unlike other larval treatments, where once your water is drained out of your containers so is your treatment, these larval chips stay in that container. When the water fills back up, they're going to continue to treat for the mosquitoes.

So this is kind of the idea behind my research. We have these little tiles that we're treating. The pyriproxyfen is treated on the tile, and then it is placed into these breeding habitats. On the left side, you'll

see some pictures of research of the environment I've set up for mosquitoes. They're little cups, and the tiles were placed in the bottom; and you can see here the larvae just swimming around it.

So when I went into my research, these are some of the ideas, based on literature, that we thought would happen when we used these tiles. For the lower doses – because we had multiple doses that we were testing – we would expect a lot of dead adults because the lower the dose, the less effective it would be at treating mosquitoes.

For the moderate doses, which are in the middle with the World Health Organization in Malawi, we would expect dead pupae. That's what we see a lot of times in these regulators is the pupae, they die before reaching that adulthood. And finally in these high doses, we thought we might see delay in development.

So the objective here of my experiment was to determine the effects of different concentrations of pyriproxyfen on various life stages of this aegis (inaudible) larvae. So for my design, we had four different doses considered of control, the 1, 3 and 10 parts per billion; that was the amount of treatment we were achieving in water. And we were treating these mosquitoes at different ages – so 24, 48, 72 and 96 hours after emergence from the eggs. We were hoping to treat first, second, third and fourth instar larvae.

We had four replicates, and we were looking for dead larvae, dead pupae, and dead adults. This data was collect at 12-hour intervals, so it was checked every 12 hours to see what had died or what had emerged. And then our analysis was a 2-way ANOVA and a Student's T test.

So just to give you an idea of how these treatments were done, 24 hours after the Aedes aegypti mosquitoes hatched from the eggs, they were separated into small little containers so that they had a sustained environment throughout the length of the experiment. These containers were treated at the different time intervals. So on this graph here, you can see on the bottom, after 12 hours, the first instar larvae were treated; and that's where it turns red. And then in the second larvae, at 48 hours they were treated; third at 72; and then fourth were treated at 96 hours.

These are the results you see. I know this is a little confusing of a graph, so let me take a minute to kind of orient you. Across the top we have the first, second, third and fourth instar larvae, what we treated (inaudible). On the left side, you'll see the percent dead; on the right, we have three different doses, the treated doses, not completing controls; and then the bottom are the hours that the experiment went through.

Really, the important thing to get out of this graph is that we saw no significant difference between when these larvae were treated. Whether they were treated at a first, a second, a third or a fourth instar larva, we saw no difference. However, the significant difference that we did see was between the doses.

As you can see here, in the first row, we'll see that there's a lot of red. The red represents dead adults, and the blue represents the dead pupae. The red, there are a lot of dead adults coming out of this lower dose, just as we had expected.

So on the graphs where you see it doesn't quite reach 100%, that's where we had even some live adults emerging from these containers whereas with the second two rows, with the third and 10-part per billion doses, we saw 100% mortality in the pupae stage. So really, if we're just looking for example at this one column, you can see that the third and the 10-part per per billion ones are working really well, whereas the 1-part per billion dose does not work very well in controlling these mosquitoes, no matter when you're treating them.

These are some pictures that I took of the mosquitoes after being treated with pyriproxyfen. As you saw with that cockroach, it has the twisted wings. Mosquitoes don't have wings as larvae, so it happens a little differently. The mosquitoes are not able to fully develop a hard exoskeleton as pupae. So you can see here in these pictures, like the top left one, it's tail paddles are all white and clear. That's where it wasn't able to develop that excess (inaudible) as well as at the (inaudible) of it.

Sometimes they can be completely white, like the bottom right picture; or sometimes they can be almost completely developed, like the bottom left. However, they still consistently are dying, especially in those last two higher doses.

We also saw some interesting things with the adults that were coming out and dying. We had multiple adults that would come out, and they would die. These are what we're seeing when these adults are dying. On the top left, you can see that that mosquito was not able to get out of his pupae, not able to fully emerge. These other two mosquitoes were also not able to fully emerge. You can see their legs are still stuck. It seemed to me that if they were able to fully get out, they would lose their legs. So pyriproxyfen can be affecting them with their legs and latter half of their bodies.

In conclusion, to come back to this chart, what we did see was at the low doses, we saw a lot of these dead adults; or we even saw live adults coming out. At those moderate doses, we saw complete dead pupae, 100%. And then at the higher doses, and this is what was different, was that we saw dead pupae. We didn't see a delayed development or the long-lasting larvae as we had expected. Instead, we saw 100% all die at pupae.

So for future research that I want to do with these larval chips now, is after treating these larval chips, I want to test different types of material. These mosquitoes, as Casey had mentioned earlier, are breeding in a multitude of breeding habitats – anything from woods, pots, to plastic to junk on the side of the road. So my hope is to treat them in this bigger material, such as metal and glass and plastic and ceramic and clay, as well as to see different water levels. The water levels in various containers are not always going to be the same, so I want to see how different water levels will affect the efficacy of these tiles.

We'd like to thank our Graduate Committee members, Dr. Koehler and Dr. Roberto Pereira, Dr. Alexander Chaskopoulou, Dr. Rebecca Baldwin and Dr. Roxanne Connelly; and our collaborators, Enrico Levi and Dr. Chris Battich, as well as the USDA, the ARS and EBCL, which funding these projects.

And I think that's it. That's all we have. If you have any questions, you can contact myself or Casey. Our e-mails are listed on this slide. And also, you can find us in the Presenter Chat Hours; we will be there to answer questions on April 13, 2016, between 1:30 p.m. and 2:30 p.m., and April 14, 2016, between 1:00 p.m. and 2:00 p.m.

Thank you.