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About the Cover: Lee Hansen, Operations Director for Pasco County Mosquito District completes this pupa as a follow-up to a previous Wingbeats cover with the adult Aedes vexans. This painting was completed with water color and acrylics. Lee has recently started dabbling with digital media and creating mixed media sculptures. His work can be seen on his Instagram @landnsee_art.

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Wing Beats has several significant changes to report to our readers. First and foremost, after serving as Editor-in-Chief of Wing Beats for 15 years, Stephen Sickerman has resigned, with the Fall 2021 issue being his last. I thank Stephen for his dedication and service in this capacity and coordinating the production of Wing Beats while keeping it relevant and interesting for the readers. I wish him the best in whatever path that follows.

Having recently retired as Director of the Pasco County Mosquito Control District and given my previous experience with Wing Beats, I was encouraged to consider this vacancy. This allows me to stay connected to the mosquito control community, attend the FMCA and AMCA meetings to promote Wing Beats, and continue with the publication that is near and dear to my heart. I have a long history with Wing Beats and have served in every capacity of the preparation, publication, and distribution of the magazine. I'm happy and humbled to report that I was selected by the FMCA board to serve as Wing Beats new Editor-in-Chief.

A Little Bit of History

A brief chronology follows to help you understand how I became involved with Wing Beats and in what capacity I served over the years;

Wing Beats was the brainchild of founding Editor Dr. Charlie Morris who served as its managing editor from the first edition in 1990 until 1995. As a University of Florida employee, Dr. Morris provided, edited, and controlled the content, but was prohibited from soliciting advertisements to financially support Wing Beats. That job fell to the original publisher of Wing Beats, David Tarver, who published a similar “Aquatics Magazine” for the Florida Aquatic Plant Management Society. David controlled the solicitation of advertisers, collection of revenues, printing, publication, and distribution of Wing Beats. Since the revenues from advertisements were not disclosed to FMCA there was strong interest in producing the magazine in-house.

There was also a desire to change the distribution policy to make it available to anyone wanting to receive Wing Beats. This resulted in an increase from 1,800 to over 4,000 copies produced and delivered nationally and internationally for each quarterly issue.

While teaching at the 1995 Dodd Short Courses, I was invited to lunch by Dr. Morris to discuss Wing Beats. After receiving several compliments, I began to suspect something was in the works. Dr. Morris proposed that I become Editor-in-Chief for Wing Beats with his assistance, since he wished to focus on other responsibilities. I was flattered by his proposal and the challenge which I accepted.

Starting with the Spring 1995 edition of Wing Beats I served as editor to train under Dr. Morris’ guidance and prepared myself to take over as Editor-in-Chief for the Spring 1996 issue. From the Spring 1996 until Winter 2002 issue, I served as Editor-in-Chief and performed the following functions: solicitation of articles and advertisements, editing, coordination and placement of advertisements and content using InDesign/Photoshop, reviewing final proofs, managing the mailing list, and working directly with the printer and the mail distributor.

Several key members of the editorial board assisted in soliciting and editing content to assure a quality publication. I attended the FMCA and AMCA annual meetings to represent and promote Wing Beats and solicit...
articles and advertisements, provided quarterly reports and budgets to the FMCA outlining income and expenses to show anticipated revenues and prepared a statement for the FMCA executive director to invoice the advertisers for each issue. Once each quarterly issue was ready for printing, I prepared a PDF file to post on the FMCA and AMCA websites for members to download. This work was all done voluntarily which was challenging during peak mosquito season.

After eight years as Editor-in-Chief in addition to my full-time duties at Lee Co. Mosquito Control District, I requested relief from some of the burden. Marin Brouillard from the Collier Mosquito Control District thankfully agreed to assume the role of Editor-in-Chief. Marin continued with these duties from the Spring 2003 to the Winter 2005 issues. I helped guide and assist Marin as the managing editor. After three years as Editor-in-Chief, Marin realized the task of performing these duties was overwhelming in addition to her full-time duties, and we decided to find a replacement. She wisely recommended a division-of-labor to reduce the workload on the Editor-in-Chief which was done in the next transition.

Stephen Sickerman served as the next Editor-in-Chief starting with the Spring 2006 issue. Since I was very familiar with working with the advertisers and their ad agencies, I volunteered to serve as the Director of Advertising, a role I filled from 2006-2012. The managing editor, Dr. Jack Peterson, solicited articles, and Marin served as the circulation editor to manage the mailing list. With our assistance, Stephen served as Editor-in-Chief until 2014, then decided to step down from the editorial desk. FMCA offered to make the position compensatory, and Stephen continued serving as Editor-in-Chief until his recent resignation with the Fall 2021 issue.

**Where We Are Today**

My first focus as your new Editor-in-Chief starting on January 1, 2022 was to review the editorial committees, the Director of Advertising, and the Circulation Editor, all of which are integral to the *Wing Beats* team. Many thanks go to those that served under Stephen and a huge thank you to those willing to continue. We do have two new editors joining our team that I am excited to have on board. I would like to welcome Dr. Randy Gaugler, distinguished professor emeritus, Rutgers University and Dr. Shelley Whitehead, senior entomologist, Manatee Co. Mosquito Control District. Of interest to note, Dr. Gaugler was on my research advisory committee during my Master’s Degree at Rutgers. Both are wonderful editors. Dr. Stan Cope was an editor during my previous tenure with *Wing Beats* and has thankfully offered to continue. I sincerely thank everyone for their willingness to help produce *Wing Beats*, while acknowledging their hard work and efforts.

Our *Wing Beats* team has been busy editing manuscripts, determining advertising, establishing contacts with the printer, refining the mailing list, etc. Unfortunately, since the Fall issue was completed and distributed mid-November, and most of our team was off several weeks during the holidays, this put the production of the Winter 2021 issue seriously behind schedule. Therefore, this current issue will be a combined issue – Winter 2021/Spring 2022 issue. This will help us get back to a realistic schedule moving forward.

**We Need Manuscripts!**

You will likely see more requests for articles and potential front covers on several social media platforms, such as the FMCA and AMCA websites, Facebook pages, and Twitter. The back of this issue includes an “Invitation to Publish” and “Author Guidelines” to encourage the submission of manuscripts and provide guidance for authors. The “Author Guidelines” have never been done for *Wing Beats* and should help both our authors and editors.

I encourage authors to share the good work you perform by submitting manuscripts to *Wing Beats*. If you’ve recently given a presentation at a meeting, consider submitting a manuscript so others may benefit. There have been some wonderful historical articles and we look forward to more. We are open to other creative ideas for manuscripts, so feel free to share your thoughts. Refer to the “Request for Articles” (pg. 50) for other suggested topics. There is no cost to you for publishing your article.

We greatly appreciate and thank those in industry who have supported *Wing Beats* through advertising and encourage your continued support.

All the best in moving forward into a new era for *Wing Beats*.

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**Dennis Moore**

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**Dennis Moore**

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Sterile Insect Technique (SIT) for Individual Property Owners: An Overview of the MosquitoMate Experience
by Karen L. Dobson, James W. Mains and Stephen L. Dobson

Purpose of the pilot trial
Traditionally, methods such as the Sterile Insect Technique (SIT) are applied by government agencies across large areas. Examples include the New World Screwworm, Mediterranean fruit fly and other economically important insects (Benedict 2021). However, a recent study demonstrated that the localized release of sterile males into a single homeowner property could reduce the *Aedes albopictus* (Asian Tiger Mosquito) mosquito population (Mains et al. 2016). Following EPA approval of *Wolbachia pipientis* as a pesticide (i.e., ‘ZAP Males,’ description below) causing cytoplasmic incompatibility and sterilizing *Ae. albopictus*, a four-year pilot study was performed in multiple Kentucky cities to examine the application of incompatible ZAP males at non-contiguous, individual properties. The goal of the study was to examine unknowns including:

1) homeowners’ willingness to pay for ZAP male mosquitoes to be repeatedly released into their properties throughout the summer,

2) whether homeowners found the presence of the male mosquitoes to be an annoyance, and

3) the perceived efficacy relative to traditional chemical approaches.

Insect flight distance is a key reason why prior SIT-type approaches have been performed over large areas. Treating large areas that exceed the typical flight range of the targeted insect can reduce the overall risk of fertile females immigrating from outside the treated area, because with large areas, immigrating fertile females can affect the perimeter only and the interior of the treated area remains out of reach. As the size of the treated area increases, the ‘at risk’ perimeter becomes a smaller proportion of the total treated area. The insect species being targeted is also an important factor because the typical flight range varies widely between insects, and *Ae. albopictus* females are thought to have a relatively small flight range (Bellini et al. 2014).

With the treatment of a single property, the entire treated area can be subject to immigrating females, i.e., because the entire area falls within the potential flight range. However, it is important to note that the size of the treated area is not necessarily limited by the specific address where the releases occur. Male mosquitoes do not respect property boundaries, and they will emigrate away from the release site as they seek mates, which can enlarge the effective treatment area and reduce the frequency of fertile females that immigrate into the treated property.

Mosquito control in the USA varies broadly by state, with some states having well-developed abatement districts. In other states, mosquito abatement can be a section within Public Health Departments, Municipal Solid Waste, etc. In Kentucky, the Department of Public Health is deployed when there is risk of disease and mosquito-borne pathogen transmission. However, homeowners seeking relief from nuisance mosquitoes often turn to private Pest Control Operators (PCOs).

The most common PCO mosquito treatment in Kentucky is ‘barrier spraying,’ in which the vegetative mosquito resting area is treated with a residual adulticide (Stoops et al. 2019). These treatments are sold as a one-time ‘knock down’ or a treatment plan that recurs throughout the summer. Some homeowners are concerned about the potential negative effects of chemical pesticides on human health and potential impacts on non-target and beneficial insects. Localized spraying can drift into neighboring yards, which can lead to negative interactions between neighbors.

Unlike traditional chemical treatments that can have an immediate knock down effect on biting adults, a pesticide that sterilizes eggs (e.g., *Wolbachia*, irradiation-based SIT) affects the next generation of mosquitoes. The impact of the males that are released today will not be observed for weeks, i.e., until the next generation matures. An egg-sterilizing approach is therefore preventative and not a quick-acting reactive approach. Therefore, our approach during the pilot trial period was to start releases in May, before tiger mosquitoes are typically observed in Kentucky. Our intent was to sterilize the earliest eclosing females, reduce the rate of population increase, and prevent the population from reaching nuisance levels later in the summer.

SIT-type approaches are species specific because the released males only mate and affect the same species. With the exception of *Ae. aegypti* (which are rare in Kentucky), *Ae. albopictus* males are not known to interact with or attempt to mate with other mosquitoes or other insects (Tripet et al. 2011). This ‘species specificity’ can be viewed as both a positive and negative. For example, ZAP male treatments do not impact other mosquito species. While we expected that a majority of
Figure 1. Examples of *Ae. albopictus* mass rearing procedures and facility. Software on computer tablets is used to track colony status and quality control.
the biting pressure and complaints in suburban Kentucky areas (e.g., Lexington, KY) was from *Ae. albopictus*, we also know that other mosquitoes co-occur in these areas (e.g., *Culex quinquefasciatus, Ae. triseriatus*, etc.). Would homeowners be satisfied with the ZAP male treatments if the non-*Ae. albopictus* mosquitoes continued to persist in their yards? This was another key question to be addressed during the pilot trial period.

Positive aspects of a species-specific approach include that it limits any effects on non-target insects, plants or other animals. Male mosquitoes cannot bite people or pets; they cannot transmit pathogens. In 2019, ZAP males were organically listed by the Organic Materials Review Institute (OMRI). During the pilot period, we repeatedly surveyed homeowners’ level of concern about beneficial insects and chemicals associated with traditional approaches, and whether these played a role in their decision to purchase ZAP males.

**Nuts and Bolts: How ZAP males are made and deployed**

ZAP males are *Ae. albopictus* mosquitoes that have been artificially infected with the wPip *Wolbachia* from *Culex pipiens* (Calvitti et al. 2010). Populations of *Ae. albopictus* in the wild carry *Wolbachia* naturally. Specifically, wild type individuals are naturally ‘superinfected’ with two types of *Wolbachia* (wAlbA and wAlbB). To create ZAP males, wild type mosquitoes are cleared of their natural *Wolbachia* infection using antibiotics, and then the ‘aposymbiotic’ line is injected with the wPip infection type (Calvitti et al. 2010). Importantly, because the wPip infection is maternally inherited from mothers to offspring (both daughters and sons), rearing more ZAP males does not require additional injections. Instead, the ZAP line is simply reared in the laboratory, and the colony maintains the infection via maternal inheritance.

In mosquitoes, *Wolbachia* causes a type of sterility known as ‘Cytoplasmic Incompatibility’ (CI). If the female and her mate have the same *Wolbachia* type, the resulting eggs hatch and the offspring are infected with the maternal *Wolbachia* type. However, when the female and male have different *Wolbachia* types, then CI occurs and the eggs do not hatch.

ZAP males in the colony are maintained similar to that of any *Ae. albopictus* colony. Because all individuals in the colony have inherited the same wPip *Wolbachia* type, the ZAP colony matings are compatible and the eggs hatch. But when ZAP males are released into the wild and they mate with naturally-infected females that have a different *Wolbachia* type; then CI occurs and the resulting eggs do not hatch (Calvitti et al. 2010).

After years of working with the EPA towards product registration, ZAP males were registered in 2017. Throughout the review, the EPA struggled with the novelty of the approach, which did not fit some of the existing required forms. The ZAP male review began within the Microbial Pesticide Branch, but in 2017 the *Wolbachia* technologies were reassigned to the newly-created Emerging Technologies Branch. With registration, the EPA issued two ZAP sub-labels: one for the traditional area-wide approach, and a second for individual homeowner applications. The primary difference between the two labels is the level of suppression, with the individual homeowner label recognizing that lower levels of suppression would result with point releases, i.e., lower relative to that which can be achieved with area-wide treatment. Both labels encourage that ZAP treatments be part of an Integrated Pest Management (IPM) approach. For example, larviciding is not expected to affect adult male mosquitoes, e.g., combining ZAP male releases with *Bacillus thuringiensis israelensis* (Bti) treatments.

ZAP males were produced in Lexington, Kentucky, where the pupae were sorted by size and then adults examined visually for any residual females (Mains et al. 2016). The ZAP males were typically released within 24 hours of eclosion, using cardboard tubes with mesh covering both ends. Previously, similar tubes have been used for interstate transport of adult male mosquitoes to CA, FL, NY and TX (Mains et al. 2019). Proprietary software was used to monitor mosquito rearing and release location (Figure 1). The same software was used to generate and send monthly pesticide application reports to homeowners, as required in Kentucky.

**Homeowner Recruitment and Return Rate**

In brief, the individual homeowner ‘Season Plan’ consisted of two treatments per week for approximately 20 weeks, beginning in early May and continuing through September (Figure 2). As applicators visited the homes, they also performed routine source reduction by emptying any containers that could serve as potential breeding sites. If permitted by the homeowner, *Bti* (Mosquito Dunks and Mosquito Bits, Summit Chemical, Baltimore, MD) was added to sites too large to manipulate. An applicator typically spent less than five minutes per home. Drive time between individual homes was the largest time component, and as described below, a ‘neighbor incentive’ was tested as a way to encourage clusters of properties to purchase the season plan at a reduced cost.

The first year of homeowner treatments was in 2018, and as part of a ‘soft opening’ strategy, we only accepted homeowners within three suburban zip codes in
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Lexington, KY. We focused on typical suburban neighborhoods using door hangers and ads targeting homeowner association magazines.

One of the most difficult aspects of recruiting homeowners was the early deadline to purchase. As described above, the season plan started well before mosquitoes reach nuisance levels in Kentucky. This meant that not only was our pesticide approach new and substantially different from traditional mosquito-control products, but we were also recruiting homeowners while there was still snow on the ground. In each year of the pilot, we did have homeowners contact us later in the summer, when the mosquito biting pressure had increased. These ‘late’ customers were encouraged to sign up early for a season plan the following summer.

The standard price for the 2018 season plan was $1,200. A ‘neighbor discount’ was offered to four (or more) adjoining properties, with the rationale that drive time between customer homes represented a substantial amount of the overall time and effort. Therefore, treating clusters of homes would reduce travel time and the relative cost per customer. However, even with the discount, the season plan was substantially more expensive than the traditional single- or multi-treatment services offered by other mosquito PCOs in Kentucky.

As we began the pilot period, we were unsure whether homeowners would be annoyed by the recurring release of thousands of male mosquitoes into their properties each week. We sent a survey to homeowners, asking questions related to satisfaction and their experience. Good response rate was achieved with the surveys, with >69% survey return rate each year.

Overall, 76.2% of customers described having fewer mosquito bites with the ZAP season plan relative to their experience prior to the ZAP season plan (n=130 responses). Of 193 survey responses, 83% described the released ZAP male mosquitoes to not be an annoyance, 10.9% were neutral, and 5.7% thought the males to be annoying. The higher cost was a concern for homeowners, with 69.8% agreeing, 25% neutral and 5.2% disagreeing to the statement that the service plan was ‘worth the cost’ (n=192 responses).

The homeowner return rate (percent of homeowners that sign up again the following year) is an important indicator of perceived product performance and homeowner satisfaction. After the 2018 season, 84% of homeowners returned to purchase a 2019 season plan. At least two of the homeowners that did not sign up were ineligible because they had moved out of the service area. The return rate in the subsequent two years was 78% and 86%, with ‘death’ and ‘customers moving outside the service area’ accounting for some of those not deciding to purchase again. The number of treated properties grew at a rate of approximately 100% each year, i.e., doubling each year. The COVID19 epidemic negatively affected activities in 2020 and 2021, but season plans were made available throughout the pandemic.

During the pilot period, two businesses purchased the season plan for mosquito control. One was a ‘dog daycare’ business, and the second was a greenhouse with an outdoor cafe that was open to the public during the summer. The dog daycare was concerned about the biting nuisance and also the potential for heartworm transmission (Gratz 2004, Spence Beaulieu and Reiskind 2019). The greenhouse/cafe owner wanted to reduce customer complaints about mosquito bites but didn’t want chemical treatments applied on site. While we were not too concerned about the dogs noticing the released ZAP males, we were concerned about the potential for customer complaints at the cafe, e.g., “Hey Waiter! There’s a mosquito in my drink!” While no major conclusions can be drawn based on two examples only, the
feared customer complaints did not occur, and both the dog daycare and greenhouse/cafe owners were pleased to see fewer biting mosquitoes. Both business owners became repeat customers and purchased the ZAP plan each year, remaining as repeat customers at the time of writing this report.

What’s next for ZAP?

When the EPA registered ZAP in 2017, it limited the registration period to five years, after which MosquitoMate would need to reapply for registration. Furthermore, ZAP registration was limited to 20 states and the District of Columbia. Several states infested with Ae. albopictus were not included in the list of registered states (e.g., Florida, Texas and additional southeastern states). Currently, MosquitoMate is working with the EPA to extend the duration of the registration and to broaden the registration for all states and USA territories that are infested or at risk of Ae. albopictus infestation. If granted, a logical next step will be to replicate the ZAP model in additional areas, e.g., in CA where Ae. albopictus is a relatively new invasive mosquito problem (Metzger et al. 2017).

MosquitoMate has substantial experience working with abatement districts in CA, NY, TX and FL, and area-wide treatment has been proven to achieve stronger suppression relative to that which can be achieved via isolated treatments of individual properties (Mains et al. 2019, Crawford et al. 2020). Therefore, a logical emphasis will be to work with abatement districts to perform area-wide treatments of problematic infestations. However, a SIT model is substantially different from the current model used by many Districts, which can be a barrier to its adoption. Cost analysis demonstrates that spreading the costs across time, the SIT approach can be cost effective relative to traditional pesticides (Benedict 2021). However, similar to infrastructure (roads, dams, etc.), much of the cost of SIT-type projects occur early, and the cost savings are not realized until the later ‘maintenance’ phases. This ‘front-loaded’ cost structure can be difficult for districts with budgets that are constrained already.

In conclusion, if the EPA approves an extended registration, the ZAP males can provide species-specific, non-chemical, non-GMO suppression of Ae. albopictus populations with efficacy that is comparable or better than that of traditional chemical insecticide treatments and without identified non-target effects. The higher cost relative to traditional chemical treatments is a homeowner concern. Furthermore, the idea of ‘buying mosquitoes to be repeatedly released in your yard throughout the summer’ is novel for many people (Figure 3). Nevertheless, the Kentucky pilot demonstrates that a majority of the homeowners that purchase the ZAP season plan do not find the male mosquitoes to be annoying, are pleased with the overall performance, and approximately 80% of homeowners return to purchase the product the following year.

References Cited


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Sentinel Chicken Coops: Even Small Changes Lead to Large Improvements

by Max Dersch

Saint Lucie County Mosquito Control’s (SLCMC) Scott Artman will likely not remember when I stopped by their newly designed, all-aluminum mobile chicken coop at the 2019 Dodd Short Course in Gainesville, but I was impressed. I fairly insisted he give me his business card so I could later request plans and a material list. At the time I was a few months into my new position with Manatee County Mosquito Control District (MCMCD) and I was hoping to make some improvements in long-practiced animal husbandry drudgery. I was encouraged by the bold redesign Scott and the team at SLCMC made (Lemire, G. 2019.) I had visions of similarly comprehensive re-designs of our eleven sentinel coops.

However, returning to our District after Dodd, I was met with a harsh reality: The MCMCD Ground Department builds things to last and our trusty, well-made (if not over-engineered) coops were only a few years into an expected eight to ten year life-span, requiring me to consider a less ambitious path to improving our coops.

While our coops were not overly burdened with problems it seemed there was room for improvement. Some of the issues we sought to improve were:

- Time and effort cleaning coops
- Old-style waterers and feeders were unsanitary and maintenance-heavy
- Feed waste via contamination and bird behavior
- Frequency of bumblefoot and/or breast keel injury as consequences of landing impacts on wire flooring
- Frequency of other infection/illness/parasites
- Time and effort addressing illness/injury

Taking stock of these issues and keeping in mind how much travel-time is required to get to our coops each week (two visits per week is minimum standard), I realized that we could make substantial improvements within the coops existing design by making a few simple changes:

- Change to different style waterers and feeders
- Coop modifications: lower perch height and replace coop flooring

The first change was replacing our old gravity-fed waterers (Figure 1a) with hanging bucket nipple waterers (Figure 1b) which immediately improved cleanliness of water, effectively sheltering it from contamination. The new hanging waterers (Premier 1 Bucket Nipple Waterer: Premier1supplies.com) also allowed for more flexibility in coop bottom construction because the coop no longer had to be kept perfectly level as it had for the old waterers.

Next we replaced the existing galvanized welded wire flooring with PVC-coated wire (Fencer-Wire: 4 ft. x 100 ft. 16-gauge black PVC coated welded wire fence, 1/2 in. x 1/2 in. openings. Homedepot.com). We found that three improvements were accomplished with the wire bottom change. First, the potential for the galvanized metal to scale apart and leave sharp burrs as it weathered was solved by the plastic coating. Second, the PVC coating effectively doubled the thickness of the wire so there was more surface area to displace the weight of the chickens’ feet. Thirdly, we found the coated wire was easier to clean than the bare metal wire.

Concurrent with the coop floor replacement we lowered the perch height from the previous 15-16 inches to a maximum of 12 inches as lowering the height along with thicker wire bottoms would further reduce the impact of landings and decrease the frequency of impact-related abrasions.

Lastly, we addressed our old-style gravity feeders, kept well beyond their useful service lives via a number of disfiguring modifications. These feeders had been reduced to sad, hideous heaps within what could only be described as tubs of failure and were as wasteful as they were unpleasant to clean (Figure 2). We discovered port-hole feeders (RentACoop Porthole DIY Feeder: Rentacoop.com and Stoltco...
Silicone Porthole DIY Feeder: Stoltco.com via Amazon) were a great solution to keeping feed sheltered from contamination while also limiting how much feed the birds could waste given they could only contact the feed with their beaks (Figure 3a). We employed a couple styles of portholes for different purposes: in particular, the silicone portholes allowed us to create space saving vertical feeders on drain pipe (Figure 3b). This was a great solution when we needed to temporarily subdivide coops during a few instances of injuriously dominant bird behavior. Our standard feeders would have occupied almost half of the aggressive bird’s allotted four square feet.

We found these improvements reduced staff cleaning time and effort and we saw a full season and a half of injury free behavior, post changes. In prior years it would not be uncommon for at least one bird per coop to develop bumblefoot infection, post changes. In our experience sheltering the water and feed from open exposure to contaminants has reduced the potential for illness and infection. As a result, we believe we have saved significant amounts of time in excess trips for extra care of sick or injured birds.

Manatee County Mosquito Control District is one of 11 programs in Florida which has been using sentinel chickens since the 1978 inception of the Florida Sentinel Chicken Arboviral Surveillance Program (Lloyd et al 2018). Until the Honey-Card Technique (Burkett-Cadena et al 2016) or other methods to monitor for arboviral disease transmission activity in an area fully eclipse the arguably primitive method of using sentinel chickens, we may well see their use for another 40 plus years in Florida. Most likely, hybrid methods will continue utilizing sentinel chickens at least until a new method’s baseline is established in the distant future.

Maintaining the safety and health of the flock while keeping them in often remote field conditions will remain an enduring challenge for those keeping sentinels. Districts employ myriad coop designs to keep the flocks safe from predators and the elements while leaving them accessible to mosquitoes. As I meet more people in our field I continue to be impressed by the ingenuity demonstrated by Mosquito Control professionals. While we may never arrive at one perfect sentinel coop plan for all programs, I am certain that regardless of design -through measures great and small- sentinel chicken coops will continue to evolve to better provide for our collective mission including the health of our flocks.

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Let’s take a moment and discuss some key precepts that relate strongly to Public Health and more narrowly, to vector-borne disease management: a proactive vs. reactive approach; cause and effect; the need to measure in order to manage. One might say these easy-to-understand ideas form the backbone of a successful Public Health program.

We’d be remiss if we did not inject another layer of realism to our discussion, however, and include other, unavoidable considerations: money, politics, and the complexity of meeting the needs of a wide range of stakeholder groups at the global, federal, state, and local level. For those who work in publicly administered public health programs, the latter group doesn’t often sync well with the former.

A case in point – VectorSurv, an online platform built by a forward-thinking team of researchers and programmers at the Barker Lab, part of the School of Veterinary Medicine at the University of California, Davis. Originally dubbed CalSurv, VectorSurv was born in 2015 through a grant partially funded by NASA. It is a sophisticated yet elegant tool that combines the power of geo-stationary satellite imaging with ground-level surveillance and intervention activities in a data repository. Mosquito abatement professionals can use it to monitor and respond to potential vector-borne disease outbreaks quickly and effectively. The application was readily adopted by California vector control districts and has since expanded to include other states such as Arizona, New Jersey, Colorado, Washington, Wyoming, Idaho, Utah, Louisiana, Tennessee, North Carolina, and the Dakotas.

The value of such a tool seems self-evident. Continually updated by all participating districts, the system allows managers to assess mosquito activity at the macro level and stay ahead of the indicators that warn of a potential outbreak of mosquito-borne illness, including West Nile Virus (WNV), dengue, Zika, chikungunya, and others. In addition to surveillance data, districts log their intervention activities including the location, rates, and timing of both larvicide and adulticide applications. The expansion of the application beyond California provided districts with the added benefit of being able to monitor activity in bordering states and counties. This interstate intelligence proved valuable in 2019, for example, when a district in Southern California was able to react quickly based on a high volume of positive WNV samples emerging in Arizona, while the rest of California remained relatively calm.

VectorSurv also offers interesting possibilities for the future. With an adequately populated data set, researchers can investigate the impact of vector-borne disease interventions in a real-world setting. In theory, combining trap counts, positive pool data, brood locations, and subsequent response activities, biostatisticians should be able to evaluate the effectiveness of mosquito control interventions – and their resultant impact on the spread of vector-borne disease – as never before. This work is still in its infancy but has incredible potential for the purposes of future planning, operations, and ultimately human health.

Putting this tool in place nationwide seems like a no-brainer, right? Not so fast. That’s where money and politics and competing demands come in. The American Mosquito Control Association (AMCA) fully supports expansion of VectorSurv into a nationwide program. No one seems to deny the value of scaling the application, the question simply becomes who’s going to pay for it.

Despite its obvious benefits and financial support from local California districts, the state, and the Mosquito and Vector Control Association of California, operational funding for VectorSurv has been an ongoing challenge. In the vector control industry, funding levels and sources can be notoriously temporal and unpredictable. And herein lies the crux of the ongoing battle vector control officials have to wage: in matters of Public Health, governments and societies tend to be painfully reactive.

The 1999 introduction of WNV into the US is one example. The outbreak brought awareness of the importance of vector control to an all-time high, resulting in an influx of new federal monies for vector control. As the specter of WNV waned, however, that level of funding continued to dwindle. In 2016, the Zika outbreak resulted in hundreds of millions in response funding administered through the Centers of Disease Control and Prevention. CDC put those monies to good use including forward-looking improvements such as increased epidemiological laboratory capacity and the establishment of five new Vector-Borne Disease Regional Centers of Excellence across the country. But again, the funding was wholly reactive and most of it, temporary. A significant increase in long-term, proactive, federal support for local vector control programs failed to materialize.

If a shift toward a more proactive approach to vector-borne disease is indeed a federal objective, permanent federal funding for VectorSurv would seem to be low-hanging fruit. The heavy lifting is already done. What remains is to promote its adoption at the national level through CDC, the National Association of County and City Health Officials (NACCHO), AMCA, and state associations. In addition to some federal monies to support ongoing administration of the program, the commitment would also require resources to conduct the prerequisite training and to provide some level of ongoing support for State Departments of Health (including US Territories and Freely Associated States) where vector control funding is already lacking.

It seems like a wise investment.

~ Public Health Landscape

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It’s no secret that East Flagler Mosquito Control District (EFMCD) is one of the pioneers in Florida’s rich mosquito control history. The district was created by the Flagler County Board of Commissioners in July 1952 to suppress the increasing numbers of breeding, biting mosquitoes. In 1990, aerial operations were introduced to further improve response during nuisance, disease, and weather-related outbreaks. Today, the EFMCD is responsible for over 84,000 acres of potential mosquito habitat, which surrounds the homes of more than 85,000 district residents. Control methods have been refined to focus on specific mosquito species, wetland mosquito management projects, and employing advanced application technology. This allows EFMCD to deliver a high level of service to the residents and visitors of Flagler County, Florida.

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East Flagler Mosquito Control District
Mark Positano – Director
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Air Operations

EFMCD deploys one 2005 Bell 206B-III equipped with interchangeable dry (granular) and liquid (ULV) application systems. To expand, operations now include a mobile UAS platform for larviciding with plans for a new helicopter underway.

EFMCD Air Crew (L to R):
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Director: Mark Positano
Senior Technician and Loadmaster: Robert Fisch
Ground Support: Jay Martin & Brian Mason

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The Annual Florida Entomologist and Biologist Roundtable 2021
by Michael T. Riles and Daniel Killingsworth

The Annual Florida Entomologist and Biologist Roundtable has been a venue for mosquito control scientists and their colleagues to discuss and present their work since 2008. Over the years, various Florida Mosquito Control Districts were selected to host this annual meeting. Normally, the meeting included morning and afternoon presentations, tours of the district facilities, and provided lunch. In most cases, the meeting was no cost to the attendees. From 2008-2013, the meeting was sponsored by the Florida Department of Agriculture and Consumer Services. In 2014, no meeting occurred. Beginning in 2015, the meeting was rejuvenated through sponsoring by the Florida Mosquito Control Association, reformatted, and started anew. Scientists from mosquito control districts, state and federal agencies, and universities interested in public health and mosquito control in Florida were the primary speakers. In 2020, no meeting occurred due to COVID-19 restrictions. The 2021 (13th meeting) meeting was virtual due to COVID concerns and was for the first time opened to participation on a national scale. This article lists the speakers and summarizes their presentations.

On 20 October 2021, the 6th Annual Florida Entomologist & Biologist Roundtable Meeting was held with great success. The meeting theme was mosquito taxonomy, identification, and surveillance challenges and innovations. Mr. David Pecor, a museum specialist from Walter Reed Army Institute of Research, was the Keynote Speaker and discussed the holdings and importance of submitting voucher specimens to the United States National Museum Collection (USNM). Mr. Pecor gave a virtual tour of the USNM and introduced the Walter Reed Biosystematics Unit and how they manage the collection for the National Museum. In emphasizing the importance of maintaining high quality collections, he pointed out several reasons to include reproducibility over time, reliable reference specimens with verifiable specimen morphology, continual availability of DNA, the capacity to model the ecology of specimens based on collection information, and creating maps associating mosquitoes with their habitat. These biological and ecological data allows for global tracking of vectors through modeling and forecasting. He spoke on the status of state-level mosquito vouchers. There is a need for specimens from regions that have reported mosquito distributions where the museum does not have a voucher specimen on record for those states (Figure 1). He called for collaboration among agencies that identify mosquitoes in the US and asked for assistance to fill voucher gaps for areas that do not have reference specimens in the National Museum collection.

You can contact Mr. David Pecor at pecord@si.edu. Please check out their website at the Walter Reed Biosystematics Unit at www.WRBU.si.edu where you can search the site for mosquito information including Vector Map (www. http://vectormap.si.edu/).

Other speakers discussed developing resources to recognize the wave of newly introduced mosquito species into the United States. Dr. Nathan Burkett-Cadena highlighted mosquito species from the neotropics and
offered predictions for future invasions into the US through Florida and other coastal states. He spoke on the importance and the need to network with fellow identifiers, the responsibility for cataloging what is in your region, and how to identify possible incoming invasive species. Recognizing mosquito species using molecular signatures and habitat designations was highlighted by Charlie Sither, a Ph.D. student from North Carolina State University. He discussed the development of mosquito species databases, including CuliciTat, a database for larval habitats, CuliciHost, a database of bloodmeal analysis, and CuliciVector, a database to analyze vector competence of mosquitoes. Computer modeling developed from his work will redefine our understanding of mosquito habitat interactions, provide insights into the dynamics of mosquito ecology, while assisting in identifying mosquito species.

Dr. William Sames, a retired US Army Medical Entomologist, presented on preparing the next generation of North American mosquito identification and

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bionomical publication. He stressed the need to update mosquito distributions at the county-level for each state so those data can be incorporated into publications. Dr. Sames discussed *Culex coronator* in the United States (Figure 3), his regional studies in Texas, and the search for *Aedes japonicus* in the southwest U.S.

Dr. Bryan Giordano discussed the mosquito BEACONS (Biodiversity Enhancement and Control of Non-native Species) working group, a southeast US collaboration of federal, state, and district stakeholders funded through the Southern Integrated Pest Management Center. Dr. Giordano’s goal is to map invasive mosquito distribution in the geographical area supported by BEACONS. You can check out the BEACONS website here: https://fmel.ifas.ufl.edu/invasivemosquito/.

Dr. Lary Reeves discussed invasive mosquitoes from a molecular identification standpoint and extended access to his resources for DNA barcoding mosquito specimens. His techniques highlight the diversity of mosquito species using molecular assays to determine the identity of cryptic species like *Culex* (Melanoconion) species and *Anopheles crucians* Complex.

A highlight of the meeting was Dr. Larry Hribar’s discussion on arthropod “by-catch” in surveillance collections and the importance of those data in contributing to our understanding of local fauna. Identifying and curating surveillance by-catch will provide information about local species and potentially establish county, state, and continental records in the US for many orders, families, and genera within Insecta.

Mike Riles presented on species record keeping and databasing in regard to county-level records for Florida and offered the first statewide report on county records for Florida since 2003.

Mr. Dan Killingsworth discussed the EntoExchange specimen sharing network, a database and website that he created for encouraging collaborations such as sharing specimens for curation, record keeping, and collaboration. Please visit the website and consider contributing to the network (https://www.entoexchange.net).
The meeting consisted of 91 attendees and 9 speakers. The speakers came from Texas (1), Maryland (1), North Carolina (1), and Florida (6). Most attendees were from Florida (68), but Washington (2), Colorado (4), California (1) Ohio (4), Virginia (3), North Carolina (1), Georgia (3), and Illinois (2) were also represented. We had one participant from the Democratic Republic of Congo. They found the meeting information on the AMCA website (Figure 4).

A demographic breakdown of title and position held by attendees showed that biologist, entomologist, and environmental specialist formed the bulk of participants (50). Supervisor/managers were also high in participation (18). The rest of the participants were described as epidemiologist (1), ecologist (1), public relations (2), students (1), sales representatives (3), surveillance coordinators (4), a Fellow of Entomology, and other (1) (Figure 4). The affiliations of participants were observed and grouped into five designations: mosquito control and abatement districts, state and federal agency, industry, and university. Mosquito control and abatement districts had the most participants (n=63). State and federal agencies totaled 19 participants. These agencies were represented by Departments of Health, the Department of Agriculture, the United States Air Force, the United States Army, and the Centers for Disease Control and Prevention. Eight industry sales representatives from Vector Disease Control International, Clarke, Leading Edge Inc., ADAPCO and Central Life Sciences participated. University participants were from NC State University (1), the University of Florida (4), and 1 professor from the University of Kinshasa, Democratic Republic of Congo.

Meeting attendance at the district level was well represented and spread over 39 agencies. Local mosquito specimen identifiers and surveillance personnel take their jobs seriously, and this was not exclusive to Florida. There was participation throughout the country with participants suggesting future topics for specimen identification within public health vector management. Personnel from federal agencies also reiterated the importance of the meeting theme and the topics discussed throughout the day. The organizers appreciated the involvement of management and supervisors from agencies as their participation level was almost as great as the biologists! Hats off to all the supervisors and managers out there that realize surveillance is a crucial part of integrated pest management and in public health. We also appreciate our industry partners for signing up and taking time out of their busy schedules to participate in discussing these important topics. The meeting was a huge success and could not have been done without the time taken and given by each speaker sharing their unique perspectives and knowledge concerning surveillance, identification, record keeping, and databasing.

The authors thank Dr. Peter Jiang for sharing the background of past meetings (2008-2013) where Dr. Jiang was the original organizer. We extend a thank you to the executive director of the FMCA for allowing use of their virtual meeting platform and running the show behind the scenes. The authors would also like to express their appreciation to Dr. Bill Sames for comments and edits prior to submission.

Stay tuned for the next meeting! If you would like to be included in the email list for upcoming information on the roundtable or to speak at this meeting, please email michael@pc-beachmosquito.com

If you have any questions regarding the topics covered in this article, please feel free to reach out to the speakers:

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The year is 2016, and the place is Miami-Dade County. The area is caught in the throes of a Zika outbreak getting worldwide attention, one impacting not just the four “hot” zones of Miami Beach, Wynwood, Little River, and Little Haiti, but the entire county. As journalists from all over the globe and as far away as Russia descended upon South Florida, millions of residents and potential tourists worried about the implications. It seemed that the disease was everywhere in an area dependent on the tourism industry.

The invasive mosquito species *Aedes aegypti* had taken root and was the cause of local transmission of a virus that wasn’t usually lethal to adults (8%), but caused severe complications in unborn children, including stillbirth, microcephaly, hearing and motor skill loss, and behavioral and social difficulties. The effects are still being studied and logged. As heartbreaking images circulated of babies born with undersized heads in Brazil, which was also experiencing a severe outbreak, panic took hold.

Some local businesses reported a 40% loss of revenue because of the resulting furor. The wedding travel industry especially took a beating. Wynwood had become THE Miami tourist destination, filled with world-class art galleries and murals, as well as outdoor bars, shops, and restaurants. Not to be left behind was the old mainstay South Beach, which had by then already enjoyed a two decades-plus run as a must-visit sun-bleached stop.

Nearby stood Port Miami, one of the world's busiest in terms of cruise line passengers, and Miami International Airport, the all-important hub at the center of the gateway to the Americas, the City of Miami.

The issue even became a political one during an election year. Local, county, state, and even federal level officials weighed in on the use aerial spraying of Naled (Dibrom), a long-term use and EPA-registered pesticide effective in knocking down adult mosquitoes. By the summer of 2016, the Florida Department of Health had begun collaborating with Miami-Dade County Mosquito Control Division officials daily to mitigate the problem. The question became what to do to break the cycle of local transmission. What kinds of safe and non-controversial mitigation efforts can be undertaken to stop this scourge? What could be done to mollify the fear of those who wanted to travel, but wouldn't because doing so could risk family-crushing side effects?

**Enter Bti**

The year is 1976 and the place is the Negev Desert in north-central Israel. Dr. Yoel Margalith, who is sometimes referred to as “Mr. Mosquito”, was busy studying the *Culex pipiens* species and happened to observe a larval die-off in a small pond. This caused him to look more closely at the contents of the water and notice the presence of *Bacillus thuringiensis* subspecies *israelensis* (**Bti**). Further study led him to discover that not only was **Bti** lethal to mosquito and black fly larvae, but it was environmentally responsible and harmless to non-targeted species such as fish, frogs, birds, pollinators, cattle, and most importantly, humans.

First used successfully during the 80s in West Africa, **Bti** was instrumental in turning back an outbreak of River Blindness brought on by blackflies. The way it works is simple: spores taken from the bacteria in the form of crystals are ingested by the mosquito larvae. Toxins within the crystals dissolve the mosquito gut, causing larvae to stop feeding within an hour. Between two to six hours later, the larvae stop moving and perish.

**Back to Miami-Dade County, Summer 2016**

“Audrey Lenhart, Ph.D., Research Entomologist of the CDC’s Entomology Branch, Division of Parasitic Diseases, and Malaria, recommended the use of **Bti** aerially sprayed in Wynwood,” says Miami-Dade County Research Manager Chalmers Vasquez, who was an integral part of the team that responded to the Zika crisis. “Correspondingly, she also recommended four aerial adulticiding applications of Naled. The decision to proceed with the recommendations was reached in consultation with the Florida Department of Agriculture and Consumer Services (FDACS).”

This combination of aerial and truck adulticiding and larviciding with **Bti** is what eventually lead Miami-Dade County to become the first community to break the cycle of local Zika transmission, positioning the County as a global leader in the fight against the virus. Not long after the one-two pesticide punches were landed, the
Centers of Disease Control began to lift their cautionary area designations, which occurred after no local cases had been confirmed for more than 45 days in a given area.

Although the virus remains in circulation in the region, and throughout the Caribbean, and Central and South America, all cases reported in Miami-Dade are now considered travel-related.

According to Vasquez, before 2016, Miami-Dade had been using Bti to control floodwater and domestic mosquitoes for decades before the Zika outbreak. These formulations included, granular, flowable, and water-dispersible granules, and the product was applied manually or by using hand-held air compressed, backpack, and hydraulic sprayers, as well as aerial applications. Helicopter larviciding treatments sporadically occurred between the mid-1980s and 2008, but against Aedes taeniorhynchus and other floodwater species, not Ae. aegypti.

Bti is commercially available as granules for use in water-holding plants such as bromeliads, and in granule and briquette form for use in outdoor pet dishes, birdbaths, rain barrels, and ponds. In agriculture, large briquettes are used in watering troughs to prevent breeding from occurring on farms.

To this day, Miami-Dade County continues to use Bti to combat mosquitoes. The formulations currently in rotation include: Fourstar® 150, Vectobac® 12AS, Vectobac G®, and Vectobac® WDB, Vectomax® WSP, and Vectoprime® FG. The formulation used in the Buffalo Turbines and hydraulic spray machines is mixed with water by highly-trained and licensed staffers, and dispersed regularly throughout the County.

Miami-Dade’s approach is multi-layered. The county combines Bti with targeted adulticide treatments as in 2016, a surveillance program that has staff monitor more than 320 mosquito traps set throughout the County every week, a robust public information and education campaign that includes in-person and virtual outreach sessions, press releases and story pitches (Figure 1), and social media messaging. Pour in mosquito larva-eating Gambusia fish and presto: integrated pest management.

Bti is hands-down the substance most used in the program, with program leaders preferring to mitigate the issue in two dimensions rather than three, getting to the vector before it can bite and fly as a preventative measure, rather than with adulticides as a reactive response. Between October of 2020 and November of 2021, more than 241,000 acres were treated with Bti to combat the invasive Ae. aegypti species and control the spread of arboviruses in Miami-Dade County.

Miami-Dade County Mosquito Control maintains a steady schedule of biweekly truck spray treatments of Bti that now includes Allapattah, Hialeah, and North Miami, among other enclaves, in addition to Miami Beach, Wynwood, and the Little River areas. During the rainy season, which coincides with peak mosquito season, routes are added to include high density and high traffic areas such as Coral Gables, South Miami, Sunny Isles Beach, the Downtown Miami/Brickell area, Coconut Grove, Little Haiti, and others. Additional routes are executed as resident complaints, high mosquito trap counts, and FDOH disease report warrant them.
The division currently uses three heavy-duty trucks outfitted with the same Buffalo Turbine machines (Figure 2) and deploys a hydraulic sprayer (Figure 3) mounted on a separate vehicle to apply Bti to pooled up standing water and hard-to-reach mangrove areas. Right hand-driving Jeeps are used to spray the larvicide into storm drains, which number in the hundreds of thousands and require constant attention.

Miami-Dade also takes steps to ensure that the droplet size and formulation are working as intended by raising mosquitoes in a lab setting. The mosquito larvae are then subject to sophisticated testing and observation using samples from droplets collected in the field. During the summer of 2019, the team even found that the larvicide scattered by the Buffalo Turbine was having the unintended consequence of killing mosquito larvae in storm drains.

To borrow Mr. Mosquito’s refrain, there is no magic bullet to stop mosquitoes, only an integrated pest management program and diverse approach. Learn more about Miami-Dade County’s Mosquito Control Division by visiting miamidade.gov/mosquito.

For more details on the County truck spray program, navigate to bit.ly/MDCTruckSpray.

Michael Mut  
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Figure 3: Hydraulic Spray Machine

ARBOVIRUS SURVEILLANCE AND MOSQUITO CONTROL WORKSHOP

AMCD 17th Annual Workshop
March 29- March 31, 2022
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CONTACT
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Vegetation is a major part of a mosquito ecosystem. Adult mosquitoes need plants for sugar and water, resting, and hiding (Xue 2008). Vegetation management is a beneficial tool for mosquito control. In St. Johns County, northeastern Florida, many residential subdivisions are isolated from older, established residential and commercial infrastructure. New developments often result in large areas of land flattened and etched in formerly densely wooded areas. As a result, these subdivisions became communities of hundreds of properties surrounded by hundreds of acres of woods and created more mosquito habitants in the process. After hurricanes Matthew in September 2016 and Hurricane Irma in October 2017 (Weaver et al. 2020) major flooding throughout St. John’s County caused mosquito outbreaks (Figure 1). This resulted in Anastasia Mosquito Control District (AMCD) receiving more than 3,000 service requests county-wide in September-October 2016 (Figure 2). During AMCD control operations, staff noted that more service requests came from the residents whose property was adjacent to the wooded area. This trend was also observed in previous studies (Weaver et al. 2013, Davidson et al. 2016).

We tested the hypothesis that there was a positive correlation between distance from the woods and the likelihood of a service request being placed. We measured the distances from each home to the edge of the woods, which would be the x-axis of our data. The y-axis represented our service request likelihood. We obtained a random list of 100 homes in each test site subdivision of residents who did not place service requests in 2016. This subset became the control group. We then compared these two groups to determine whether there was a correlation between the two variables.

We randomly selected properties for the control by obtaining property addresses for each subdivision from the St. John’s County Property Appraiser’s office. This list was exported to an Excel spread sheet. The Samara Lakes subdivision spread sheet created a list of 878 individual properties in the subdivision. An online web app called Research Randomizer generated a subset of 100 random numbers ranging from 1 to 878. AMCD matched these numbers with the addresses listed in their corresponding rows and this became our new subset list of randomized control properties. Properties listed under any category other than “single-family residential” were excluded from our study, as were any properties which were not fully developed at the time of the hurricane. For each property removed from the list, a new number was generated.
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and the process was repeated until we had a full list of 100 randomized properties. This was repeated for all three subdivisions.

For measuring the distances from the wood edges to the home sites property line we used Google Maps which contains a distance-measuring tool. The measurements were then verified by driving to the three subdivisions and manually measuring the distance with a surveyor’s wheel. A wooded area was defined as any area one square acre or larger of dense vegetation through which the ground could not be seen by aerial view.

Since some of the subdivisions were less than 5 years old, the exact location of the properties were not yet pinpointed on Google Maps. Additional trips were needed to drive through these neighborhoods to record the relative locations of each property. This step had to be repeated several times, especially in instances when we discovered the property was still under development as seen by visual inspections. This process significantly increased the time taken to generate the list.

The data showed that the average property with a service request was approximately half the distance from the woods as the average property without a service request (Figure 3). Additionally, there was a significantly lower percentage of service requests placed from properties in the upper distance extremes. The study process was conducted in two other subdivisions (Glen St. Johns and Sevilla). The results showed similar conclusions to those in Samara Lakes subdivision. The result is also similar to the report about vegetation coverage related to service requests (Davidson et al 2016).

In conclusion, our study revealed a strong negative correlation between a property’s distance from the surrounding wooded areas and the likeliness of a service request call. This study will help mosquito control and disaster relief workers: (1) to determine areas of need and treatment when considering designing and implementing a mosquito control strategy, and (2) could be used in planning for barrier spraying or buffer zone treatment around the subdivisions by ground or aerial application.

**REFERENCE CITED**


This research was supported by CDC grant 1U01CK000510-01: Southeastern Regional Center of Excellence in Vector-Borne Diseases: the Gateway Program. We thank Christopher Bibbs and other AMCD staff for support and help during this study.
Finally! After 10 years of public information meetings, debates both local and international, a countywide referendum, and fifteen webinars, we have finally released the Oxitec genetically modified male *Aedes aegypti* mosquito in the Florida Keys.

It has been an illuminating journey to get to this point, and there is a lot of information available to read to get all the details (see keysmosquitoproject.com for all Oxitec/FKMCD project information), but this article is going to focus more on what it has been like to actually be a part of the release and all the cool things we’ve learned since we’ve started this project.

We should first bring the reader up to speed. In April 2020, at the start of the COVID-19 pandemic, Oxitec received an experimental use permit (EUP) from the Environmental Protection Agency (EPA) to release their modified male mosquitoes in the Florida Keys. This was followed by regulatory approval for the project by the Florida Department of Agriculture and Consumer Services (FDACS) in June of 2020. Then, it was up to the Florida Keys Mosquito Control Board of Commissioners to vote on the project. In August 2020, the FKMCD Board voted 4-1 to approve the Oxitec project for 2021.

**OX5034**

Oxitec hit the ground running and immediately started hiring a US-based crew since international travel was restricted due to the pandemic. They also modified two lab spaces in the FKMCD Marathon headquarters for their requirements for the new OX5034 strain instead of the previous OX513A strain. The OX5034 strain is shipped to the Florida Keys in small plastic containers as eggs. Each egg container is placed in a small rearing release box in the field with water and food to hatch and grow under local environmental conditions, just like our local wild-type *Ae aegypti* population. The female OX5034 larvae die as early instars while males emerge as adults and go in search of wild female *Ae aegypti* for mating. Shipping the OX5034 mosquitoes as eggs was a game changer for the Oxitec team because this meant less room required for rearing, removal of the requirement to rear the mosquitoes with tetracycline and eliminating a number of operational requirements.

**Projects and their Objectives**

There were two EUP projects for this trial. Project A was a single-point deployment of the rearing box (release box), and Project B was a multi-point deployment. Project A began first and had about 12 weeks of releases and overlapped with the start of Project B, which had around 16 weeks of releases. Both A & B treatment sites were conducted in different areas of the Keys. All sites were chosen based on a comparable human population density, the presence of *Ae aegypti*, and area size. Control sites were chosen using the same metrics. There was a period of pre-release monitoring for both trials to ensure adequate *Ae aegypti* presence as well as 10 weeks of post-release monitoring to document any residual activity of the Oxitec transgene in the environment.

The objectives for Project A were to determine the duration of effect, male flight range and longevity, the percent mortality of female larvae, and percent of the wild population treated. The objectives for trial B were the same but with multiple release points in a zone. To measure these objectives, we placed BG Sentinel traps® (BGS) (Biogents, Regensburg, Germany) and ovicups within each release zone. The BGS traps were powered with A/C current and the proprietary BG Lure was used as the attractant. Nets were collected once per week. The ovicups were refreshed with tap water once per week and used hardboard cut into rectangular paddles as the egg substrate (Figure 1). Six treatment areas plus three control areas each with numerous BGS traps and ovicups equates to a lot of nets and paddles to manage. FKMCD and Oxitec deployed in teams of two to make door-to-door visits to every home in the Release and Control areas, to talk with residents about the project, and ask if they would be interested in hosting a trap or a release box.

Initially, there was some apprehension from teams approaching residents about the project. While we trained for public engagement including a workshop with the Monroe County...
Sheriff’s Office on de-escalation techniques, the support from homeowners was overwhelming. Residents were eager to help the project by hosting boxes or traps.

**Release boxes**

The placement of release boxes required special consideration. All release boxes were placed behind fences and locked gates, out of view from the street. We did not disclose the locations of the release boxes to the public to protect the resident’s privacy.

Each release box was a hexagonal box that consisted of a plastic cup containing OX5034 eggs (the females of which would die as early instar larvae) shipped from the United Kingdom Oxitec production unit (Figure 2a). The release box would also contain prepared larval diet, a water preservative, and tap water. There were several openings along the top of the release box where the males would exit as adults (Figure 2b).

It was estimated to take 10 days to rear the Oxitec males to adulthood in the Florida Keys environment. To ensure we could hit the required target dose, more release boxes were placed than were expected to be used. Once performance had been checked during the development stages, any additional release boxes that were not required could be discarded. To measure whether the release boxes were releasing the estimated number of males, we also set out release boxes that were enclosed in butterfly cages so mosquitoes could emerge from the box but would be trapped in the cage (Figure 3). These cages would be aspirated, and the recovered mosquitoes would be triple-contained, returned to the lab, frozen, and counted to determine the release box yield.

In the last week of April 2021, FKMCD and Oxitec officially deployed the first boxes for the release of OX5034 males. Although there was little fanfare, it...
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<th>Traditional Spray System</th>
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<tr>
<td>4 hours</td>
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<td>40 hours</td>
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<td>1</td>
<td>Aircraft deployed for mission</td>
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<tr>
<td>300 gallons</td>
<td>Gallons of insecticide needed</td>
<td>1,291 gallons</td>
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*Patent numbers: 6886784, 7004431, 13506*
was rewarding to be a part of this historic moment (Figure 4).

**Data Collection and Management**

With a large number of traps, ovicups and release boxes to monitor each week, we needed a concise and manageable data recording system. Oxitec used ArcGIS (Esri, Redlands, CA) Field Maps and Survey123 apps to gather and store this information. The software was user-friendly and made obtaining data fast and easy. A unique QR code was assigned for each trap, ovicup paddle, release box, egg batch, and diet batch, allowing analysts to trace back recordings to specific points. The QR codes were scanned with a phone which eliminated human writing and typing errors. These apps were extremely useful and certainly something FKMCD will look into for future fieldwork.

**In the Lab**

Once the pre-trial data were collected and release boxes were deployed, all we had to do was wait for the Oxitec males to mate with the wild females. Of course, there was no actual downtime. There was plenty of field work involved in collecting the BGS nets and ovicup paddles. Once collected, paddles were dried and eggs were counted. Paddles were laid in individual slots within a tray and flooded to induce hatching. The resulting 1st instars were counted and separated individually into water droplets on a petri dish. Each larva was observed under a fluorescent microscope and scored for fluorescence.

**Figure 5: Photographs of mixture of Oxitec (1) and wild-type (2) first instar larvae under fluorescent scope (A), OX5034 larvae (B), and Wild-type L1 with auto-fluorescence (C).**

Being able to distinguish between fluorescent and non-fluorescent larvae is an important skill to have for this project. The fluorescent gene is passed on with the self-limiting gene from OX5034 males to their offspring. When we see fluorescent larvae, we know that the OX5034 males have successfully mated with wild females. It is not difficult to tell the difference between the two because in a side-by-side comparison, the Oxitec larvae light up like Christmas trees (Figure 5A). However, we didn’t realize that wild-type *Ae aegypti* midguts can auto-fluoresce, although this is well-known in the fluorescent microscopy community (Kliot & Ghanim 2016). This confounder can be easily eliminated by screening the larvae as early instars. Techs had to undergo intense fluorescence identification screening to ensure we could accurately differentiate the Oxitec strain from...
wild-type larvae, including filtering out any auto-fluorescence in the midgut of wild-type larvae.

After correctly identifying the larvae, we separated them into rearing cups by strain, location, and collection date. We fed and reared them to adulthood. This is when they would go through another screening process to determine whether the eggs belonged to wild *Ae aegypti* or *Ae albopictus* (we did keep an eye out for other container species) and importantly make sure that all of the Oxitec mosquitoes were male (no females have been detected as of this writing). Samples were also routinely sent for molecular confirmation of the fluorescence screening.

Just when we were collecting data like a well-oiled machine, Tropical Storm Elsa made her way towards The Keys. As per the protocols agreed with regulators at the start of the project, if a named storm is expected in the release area, all BGS traps and release boxes must be removed. It was disappointing, but we knew this was a possibility in our hurricane-susceptible area. Tropical Storm Elsa was not very destructive, so immediately after the storm, we redeployed the traps and release boxes. Oxitec extended the timeline for Project A and delayed the start of Project B accordingly. In the meantime, we continued to collect paddles and it was evident that the OX5034 males were mating with wild-females because we found fluorescent larvae in the project areas. It was gratifying to see those larvae, and to know the product was working.

In addition to the BGS and ovicups, we will also be collecting larval samples from the release areas to check if mated females are also laying in natural breeding sites. Post-release monitoring of the Oxitec mosquitoes via ovicups will likely take us into February 2022. By then, we will have all the data gleaned from these particular projects and may be considering how to best use the Oxitec mosquitoes for operational control of *Ae aegypti* in the Florida Keys.

**Acknowledgements**

We thank the FKMCD and Oxitec staff who are working to make this ambitious project a reality (Figure 6).

**References Cited**

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Social media: you either love it or you hate it! Still, social media is a critical tool for public education in mosquito control. It helps us communicate important information to residents in the form of public service announcements, treatment alerts, mosquito-borne illness advisories, etc. Reaching out to newspapers or radio stations is helpful, but there is no guarantee that your message will be relayed exactly the way you want. Using social media ensures that you are in control of how you reach your stakeholders.

At Citrus County Mosquito Control, we choose to use Facebook and Twitter as our social media platforms. Our population is largely made up of adults 25 and older with a majority over 65 years of age who are very active on Facebook. We use Twitter to network with our mosquito control industry members, and to send quick alerts out to residents because they can be notified every time we tweet. Facebook is effective for sending general announcements and getting residents real-time, up to date information. It allows us to show our community an exclusive look at what we do, and helps us engage directly with those we serve. The District posts on Facebook once or twice per week. Posting too often may lead followers to unfollow/unlike our page. We have increased our following by encouraging people to share our posts with their friends.

Successful Facebook posts are vibrant, show our employees, and provide a behind-the-scenes look at our District operations. We show our residents that we are more than just a fog truck, and they enjoy seeing that. Another good practice is to keep residents informed about the timing of ULV truck and helicopter spraying. We take a different approach for Twitter, where we post around three times per week. Our mission statement included in our bio has a colorful banner to attract more followers. Our District page includes links to other mosquito control districts, industry professionals, and researchers worldwide. Through tweeting often, and using hashtags, we have gained 22 new followers per month since March 2021.

Successful Twitter posts can educate and entertain an audience. When posting, ask questions and use a few relevant emojis and hashtags to encourage participation and reach more people. Avoid posting stock photos that look like ads as people just scroll past them.

Bottom line... post things that people want to see, not things that...
Before and After Our Twitter Makeover

**Before**

Before and after pictures showing the Citrus Mosquito account before and after a makeover.

**After**

Examples of Successful Twitter Posts

Two examples of successful Twitter posts are shown:

1. A call to action to make them want to keep scrolling. If coworkers do not want to be photographed, then get in the scene yourself and ask someone to take a photo. Be sure to check socials daily and reply to residents that comment on posts; it increases transparency, opens communication lines, and keeps everyone happy.

2. Please visit our website at citrusmosquito.org to learn more and follow us on Facebook and Twitter @citrusmosquito for updates.

Tarolyn Frisbie

tfrisbie@citrusmosquito.org

Public Education Specialist

Citrus County Mosquito Control District

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Request for Articles and Author Guidelines
by Dennis Moore ~ Editor-in-Chief

Invitation to publish in Wing Beats

Wing Beats is an international publication for mosquito and vector control produced by the Florida Mosquito Control Association (FMCA) and is an official publication of the American Mosquito Control Association (AMCA). The publication is supported by advertisers and is mailed to over 4,000 individuals. Wing Beats is also available online for AMCA and FMCA members.

Intent and Purpose of Wing Beats

Wing Beats is published quarterly and is written and produced primarily by operational personnel. Our goals are to:
1. keep interested parties informed on matters related to mosquito and vector control.
2. disseminate information to educate and raise the level of the mosquito control profession.

Request for Materials

Wing Beats seeks interesting technical and field-related articles about mosquitoes, mosquito control and other vector related topics. The articles need not be “scientific” in nature and are often just a page or two. However, longer articles tend to be the most interesting and are encouraged if the additional space is warranted. We invite you to submit vector related articles so that our readers may benefit from your experiences. We strongly recommend that you ask your colleagues to review the manuscript prior to submission.

Wing Beats is a forum for sharing viewpoints on vector topics, information on operational advancements, improvements on application or surveillance equipment and legislative issues that affect our profession. This less formal medium is well suited for the expression of ideas and stimulating readers to think about potential challenges in our industry as they evolve. We also welcome manuscripts from the academic community to share your important research. Much of this information is publishable but may not fit the format of a refereed scientific journal.

Have you heard an interesting presentation recently at your state or regional meetings? If so, ask the presenter if they would be willing to submit their presentation to Wing Beats for publication so that a larger audience can benefit from their experience and expertise.

In addition to our regular articles, following are a few examples of mosquito related topics we are looking for:

- Emerging mosquito control technologies
- New products in the pipeline, industry updates
- How are you using social media
- Taking mosquito control to the classroom
- Legends of mosquito control - via nomination
- Highlighting MCD’s program anniversaries
- Tick update: new threats, new control measures
- Mosquito control in China, Europe, Australia, etc.
- Status of Wolbachia, SIT, genetic modification
- More manuscripts from the academic folks - please!
- Interactions with your local media networks
- Evolution of mosquito trap technology
- How are you using 3-D printing
- The future of AI and mosquito control counting, sorting, IDing, sexing
- Articles from military members
- Update on hand-held devices for surveillance data management - the move to paperless offices
- Use of new mapping technologies
- Use of unmanned aircraft systems (UAS)
- Impact of global climate change on mosquito-borne diseases
Author Guidelines

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Send your manuscripts in Microsoft Word format electronically to Dennis Moore at dmtrinity@outlook.com. If you have photographs, tables, or graphs, send them as separate files in addition to placing them in the Word document for best reproduction. Graphics should be in tiff, jpg or pdf file format (>300 dpi). Each figure and table should be clearly numbered and titled and be self-explanatory.

Title Page and Author Information
The manuscript title page should include a suggested title, the first and last name and the affiliation of each author, email address of each author, and address and phone number of the primary author.

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- Figures should be submitted as high resolution tiff, jpg, or pdf files.
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- Always spell out numbers when they appear as the first word in a sentence.
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- Publication titles (not article titles) are in italic type.

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* Author AB, Author CD, Author EF. Publication year. Article title. Journal Title (Using Biosis abbreviation) 00:0000–0000.

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Snake River Canyon looking south to Twin Falls, Idaho. Home of Twin Falls County Pest Abatement District and the nearby Frontier Precision office in Jerome.

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