FLIRTING WITH THE ASSASSIN

With insects growing resistant to insecticides, and climate change creating a bug boom, towns consider a lab-bred mosquito with a killer gene.

BY **LAUREN J. YOUNG**
PHOTOGRAPHS BY **RYAN YOUNG**

From left: A female *Aedes aegypti* captured in the wilds of California; Bryan Ruiz, a mosquito tech, inspects a home for mosquito larvae.

IN THE NORTHWESTERN outskirts of Visalia in Tulare County, California, Bryan Ruiz drives down a familiar dirt road that cuts through farmland. He comes up to an irrigation pipe that's created a "pretty nasty" situation—a small patch of vegetation and algae-covered water baking under the early June sun. As his shadow looms over the pool, a wormlike critter less than half an inch long quickly tries to submerge out of sight, but before it can, Ruiz scoops it up with a long metal dipper. He squints at his catch: a larva of *Culex*, a genus that includes common house mosquitoes.

While it may seem innocent at this phase, even a bit clumsy and silly tumbling in the water alongside perhaps hundreds more wrigglers, the larvae grow up to be disease-carrying bloodsuckers—what scientists call vectors. So Ruiz flags the spot for treatment with bacterial spray toxic only to the insects. This is a typical day for Ruiz, a technician at Delta Mosquito and Vector Control District (DMVCD): hunting for an insect that also hunts for him.

Tulare County, located in the agricultural center of California, the San

Joaquin Valley, has long battled the pest. DMVCD, an independent abatement district, was founded in 1922 with a push from the Visalia Women's Club, when mosquito-borne malaria once ravaged the region. Today, a new threat menaces the 712-square-mile region, known for its dairy farms, citrus orchards, and vineyards surrounded by growing development. *Aedes aegypti*—an invasive mosquito species capable of spreading the Zika virus, dengue, chikungunya, and yellow fever—has expanded its reach in California by making itself at home in anything from dog bowls to small toys left out under sprinklers. Ruiz's job has become even trickier as *Ae. aegypti* evolves to evade almost all conventional control tactics aimed at adult skeeters. "Most of the stuff we have doesn't affect them," he says.

With insecticide resistance increasing and climate change priming the environment for longer breeding seasons and a wider geographic range, the DMVCD and state biologists are concerned the area might see a bug boom. "Before, we haven't really had the *Ae. aegypti* population," says

The DMVCD team puts out two kinds of traps in neighborhoods during summer. The blue one is used for blood-hunting species like *Ae. aegypti*.

Crystal Grippin, the biologist and scientific program manager who coordinates surveillance of the insect at DMVCD. "Now we're getting multiple locations with 10 female *Ae. aegypti* per trap." (Only female mosquitoes draw blood; males consume nectar and fruit juice.)

Local abatement units like DMVCD and international companies alike are seeking new ways to combat the pests. The biotech company Oxitec has advanced one of the more novel—and controversial—approaches to curbing *Ae. aegypti*: releasing more *Ae. aegypti*. But theirs are no ordinary mosquitoes. These are non-biting males engineered to carry a time-bomb gene that passes on to offspring and kills females in the larval stage. "It is 100 percent fatal to female larvae carrying this gene," says Rajeev Vaidyanathan, an entomologist and director of US programs at Oxitec. As the population drops, so does the risk of disease.

Oxitec hopes to make Tulare County the next site where it will test out its strategy. After the company showed promising results from a 2021 pilot project in Florida, the US Environmental Protection Agency cleared it in March 2022 to do a second trial in the state and to see how it does in Central California's vastly different climate. Officials at the California Department of Pesticide Regulation are reviewing the firm's permit request, which, if approved, might mean the trial would start moving forward in spring 2023.

While Oxitec's invention is trademarked as "Friendly" mosquitoes, not everyone is charmed by the genetically tweaked insects making a buzz in the neighborhood. National and local groups have complained about

the state's review process and the company's approach to consulting and communicating with residents.

If the Tulare County trial moves forward, though, it could aid DMVCD techs like Ruiz by taking down the swarm from within. While his agency awaits the state's decision, it is working with Oxitec, providing a much-needed community connection and history of the local landscape. "I'm really excited and lucky to be collaborating with Oxitec," says Mustapha Debboun, a medical and veterinary entomologist and DMVCD's general manager. "I'm always interested in seeking additional techniques that would work."

IT'S A BIT of a surprise that these black-and-white insects have been able to survive in the arid landscape of Tulare County, given their tropical beginnings. *Ae. aegypti* originated as a forest mosquito, supposedly in Africa, before a strain of the species spun out across the globe when humans began to settle in villages and store water in containers. Their arrival in the Americas aboard slave-trading ships in the 17th century brought outbreaks of yellow fever, and in more recent times, they have caused other viral infections: chikungunya and dengue, known for causing fever and joint pain, and Zika, which can trigger birth defects in the children of infected pregnant people.

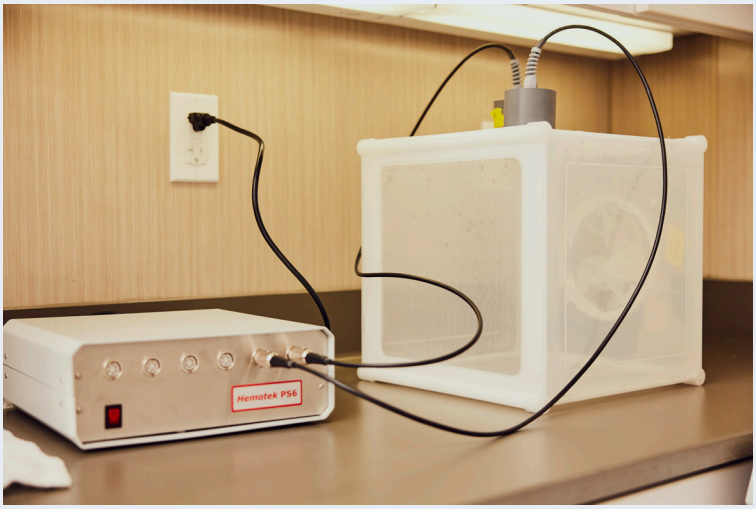
In the DMVCD lab, biologists identify and tally up mosquito species collected from 90 different traps at urban, suburban, and rural sites.

The species largely stuck to more tropical areas of North and Latin America for about a century before migrating northward, their eggs or larvae often hitching a ride with humans. Then, in 2013, health officials detected *Ae. aegypti* in California. As of July 2022, 22 of the state's 58 counties have reported the mosquitoes' presence to the California Department of Public Health. (They've also been spied as far north as Ontario, Canada.)

That spread is partly influenced by climate change, says Erin Mordecai, an infectious disease ecologist at Stanford University. Mosquitoes are ectotherms, meaning they're dependent on external sources of heat. "Every life cycle process they go through," she says, "is dependent on temperature." A warmer environment speeds up their life cycle, so they reach adult stages faster, have more offspring over a longer breeding season, and bite more humans. To avoid the drier triple-digit heat that would otherwise desiccate their bodies, *Ae. aegypti* has begun sheltering indoors and in the shade, and DMVCD's mosquito season now typically begins in April and lasts until November.

A longer season and a bigger population increase the risk to humans. While California has reported travel-associated cases of dengue, chikungunya, and Zika, there haven't been any known instances of *Ae. aegypti* spreading the diseases in the state—but they could. In 2018, *Ae. aegypti* was the fifth most prevalent species detected by DMVCD, with 2,129 nabbed in surveillance traps; in 2021, the agency caught 16,450, making it the second most abundant species in the district.

To gauge local populations, the agency uses traps that cater to the likings of target species. The one used to fool *Ae. aegypti* is the BG-Sentinel, a laundry hamper–like cylinder that lures the bug with CO_2 emitted from a sugar-yeast solution and a scent similar to dirty jeans wafting from a tube of pellets. "I don't even smell it anymore," says DMVCD's Grippin. A small motorized fan in the contraption then sucks up unsuspecting females hungry for a blood feast. Laboratory technicians set about 90 devices baited for some 16 mosquito varieties every day, tucking them in the shade of bushes in residential front yards and parks. Some units can collect more than 4,000 skeeters by the time they're picked up the next morning.



Blood is warmed and delivered to a breeding chamber for *Culex quinquefasciatus*, a vector for West Nile virus. DMVCD tests insecticides on them.

To push down mosquito numbers, the district tries to stop them when they are young and most vulnerable. First, the team urges locals to eliminate standing water. But the microscopic black eggs of *Ae. aegypti* are easily mistaken for mold, dirt, even shaving stubble, Grippin says. Simply emptying containers doesn't always work, because eggs can survive without moisture for up to a year. "Once you put more water in, they hatch," Ruiz says. He recommends deep scrubbing and repeated checkups.

For larvae, team members deploy biological methods like the use of *Gambusia affinis*, commonly called mosquitofish. These freshwater swimmers are easy to breed and maintain, so the district keeps a large hatchery where residents can pick them up for free. If fish aren't an option, the squirmers can be treated with natural larvicides, such as *Bacillus thuringiensis israelensis*, in water.

If DMVCD detects an abundance of adult mosquitoes or the presence of pathogens in them, technicians apply chemical fogging treatments before dawn, when fewer people are outside. Ruiz says it's a last resort. "Our main objective is to not use chemicals at all."

The compounds might not even do the trick. Decades of pesticide overuse have given mosquitoes time to develop immunity. In 2020, researchers from the California Department of Public Health published a study on *Ae. aegypti* in the state that pointed to the possibility of the

species developing resistance to pyrethroids, a group of commonly used insecticides. DMVCD's own tests on certain lab-born and wild-caught species have shown resistance to the compounds as well.

With an eye toward finding alternative ways to thwart *Ae. aegypti*, in 2022 DMVCD was selected by Oxitec from more than 10 other districts in California to host a test of the company's genetic assassins. Hot off its first trial in the US, Oxitec was looking to expand and see how its mosquitoes would fare in a more arid environment. The dry heat of Tulare County was just right. "I describe Visalia as kind of our Goldilocks spot," says Oxitec US program director Vaidyanathan. "It has high numbers of *Aedes aegypti*. ... It's small enough that we can get all over Visalia in a way that we would not be able to in Los Angeles. It also has a very supportive mosquito and vector control district in Delta."

Officials in Visalia see a potential panacea in the firm's novel approach. "The technique that Oxitec has is very ingenious," says DMVCD general manager Debboun. "I think this will work."

IN MAY 2022, colorful weatherproofed containers about the size of rice cookers began dotting yards in the Florida Keys. But popping open the lid and pouring in water didn't result in steamy, fluffy grains. Part of Oxitec's second round of releases, these vessels will incubate some 7 million eggs of the company's lab-bred male *Ae. aegypti*. The bugs hatch and emerge in about 10 days to seek out females within a few hundred feet and share the killer gene that Oxitec's been developing for two decades.

DMVCD staff swing by dairy manure pits to spread a bacteria-based biocontrol agent that targets mosquito larvae. The treatment works on *Ae. aegypti* with proper application.

The firm was founded in 2002 with the support of Oxford University in the United Kingdom, where the headquarters and research and development facilities of the now-US-owned company remain based. From the start, Oxitec focused on developing scalable genetic technologies that would squash harmful insect populations—from crop pests like the soybean looper caterpillar to disease-carrying vectors like *Ae. aegypti*. Oxitec's solution is a technique it calls RIDL: Release of Insects with a Dominant Lethal. In other words, company scientists have been fine-tuning a genetic time bomb fatal to targeted insects.

It all began with Oxitec's very own engineered strain of male *Ae. aegypti*, called OX513A. While they don't look any different from their counterparts in the wild, these lab-reared specimens are genetically designed Trojan horses that carry two genes—one that identifies their offspring under UV light and another that spells demise for any female progeny. In the lab,

the insects are fed tetracycline, an antibiotic that functions as a lock that stops the killer gene from flipping the death switch so the skeeters can reproduce in captivity. But once Oxitec frees the bugs, the gene turns back on in the absence of the drug. (Modern agriculture does tap tetracycline, and while a 2022 EPA statement noted the possibility of the mosquitoes being exposed to sufficient amounts is "remote," it said the company could not release its males within 500 meters of certain enterprises that use the drug.) After Oxitec males successfully mate—passing on the gender-targeting lethal gene—the wild *Ae. aegypti* females go on to lay viable eggs, but their female larvae never make it to bloodthirsty adulthood.

Since *Ae. aegypti* males mate only with females of the same species, Oxitec says its approach shouldn't have an impact on the overall diversity of the world's more than 3,500 other mosquito species. Omar Akbari, a molecular biologist who studies the genetics of mosquitoes at the University of California San Diego, and is working with his own team to engineer a sterile male *Ae. aegypti*, says that Oxitec's process could help reduce the overuse of insecticides. "In a lot of ways, I would view it as a green technology," Akbari says. "I would argue it is a great approach, a safe approach."

Starting in 2009, Oxitec conducted trials in Grand Cayman and Malaysia in 2010, Panama in 2014, and releases in Brazil since 2011. The firm reported that targeted areas with wild *Ae. aegypti* saw up to a 95 percent drop in population numbers.

COURTESY OXITEC

For its pilot project in the Florida Keys, Oxitec set out "just-add-water" boxes with genetically engineered male larvae of *Ae. aegypti*.

Despite these apparent successes, the technology sparked criticism. In 2019, an article published in *Scientific Reports* found that Oxitec males bred with local *Ae. aegypti* in a city in the Brazilian state of Bahia resulted in hybrid female mosquitoes. The authors pointed out that it is unclear how this may affect disease transmission or other control efforts. Shortly after, Oxitec responded that the paper had identified no unanticipated effects. UC San Diego's Akbari says the findings actually show that the hybrids with Oxitec genes didn't persist in the population, and *Scientific Reports* eventually noted editorial concerns over some of the authors' claims and interpretations of the data. Nonetheless, opponents of the company's real-world experiments still cite it as evidence.

Oxitec's first US trial faced a long road before the EPA gave the company a green light to let its second-generation *Ae. aegypti*, OX5034, take wing in 2021, in partnership with the Florida Keys Mosquito Control District (FKMCD). The application was passed to two regulatory agencies before landing with the EPA in 2017, kicking off the whole review process.

Controversial from the start, the plan drew 448 responses during the agency's public comment period. A state agency also had to weigh in. "In the meantime, it was extremely important to try to educate the public on this project," says Andrea Leal, executive director of FKMCD. "It's not a very simple thing to explain to folks."

While data from the 2021 release has not been peer-reviewed, Oxitec reported that every single larva that matured became an adult male and all the blood-hunting females died. The EPA has since allowed the company to continue its experiment in the Keys, where it aims to reiterate its data from the first trial on a larger scale, all while moving forward in California. Oxitec stated that the mosquitoes used in Florida wouldn't be given tetracycline at any stage, meaning the killer gene should work as intended on the female offspring.

On farms around Tulare County, *Ae. aegypti* breeds in animal water troughs and drinking bowls, while other invasive mosquitoes thrive in the fields and fruit trees.

On the ground in California, Oxitec's permitting process has also been bumpy. National and local groups took issue with the review process. Organizations like the Center for Food Safety and Friends of the Earth noted that Tulare County covers more than 4,800 square miles, yet the locations of proposed releases have not been disclosed, so residents have no way to know if the project would directly affect them.

As part of its review of Oxitec's application, the California Department of Pesticide Regulation (CDPR) held a 15-day public comment period in April 2022. "Fifteen days is simply not enough for something that is new to this area," says Tulare County native Ángel García, co-director of Californians for Pesticide Reform. Garcia adds that the CDPR did not adequately attempt to notify farm-working families about the feedback period, and national groups also cited the brief window. In its announcement of the public comment period, the CDPR listed only an email address for feedback. A CDPR spokesperson, responding to *Popular Science*'s request for comment, stated that the department is working to provide additional opportunities for public input, including a second comment period that will be announced at a later date. The spokesperson noted that the CDPR always takes public comment by phone, email, or letter and that this would be clearly stated in its next communication with the media, stakeholders, and the public.

At DMVCD's monthly board meeting in May, about a dozen farmworkers living and working in Tulare County, a community that has wrestled with exposure to agricultural pesticides, voiced concerns about an Oxitec release and gaps in the firm's communication. Some held signs, one of

which was marked in Spanish: "*#No somos ratas de laboratorios*"—"We are not lab rats." Cecilia Andrade, secretary of a local group affiliated with Californians for Pesticide Reform, who was there, points out that many rural residents, including her, do not have robust internet connections or any service at all, making it difficult for them to learn about Oxitec's plan. "A lot of the information references the website, but a lot of people in this community don't go to the website to get their information," she says.

Oxitec responded to *Popular Science* via email that all its community outreach materials and web content are available now in English and Spanish. It stated it has been canvassing door to door, distributing informational flyers, pushing to social media, rolling out booths at local farmers markets and events, and hosting monthly webinars, some in collaboration with DMVCD. "We've probably knocked all together on thousands of doors," says Oxitec US programs director Vaidyanathan. "The real story is that people are overwhelmingly well informed and supportive of this technology. In both Florida and California, we typically get two to three times as many people signed up to host [release boxes] than we actually need."

"I DESCRIBE VISALIA AS KIND OF OUR GOLDILOCKS SPOT. IT'S SMALL ENOUGH THAT WE CAN GET ALL OVER VISALIA IN A WAY THAT WE WOULD NOT BE ABLE TO IN LOS ANGELES. IT ALSO HAS A VERY SUPPORTIVE MOSQUITO AND VECTOR CONTROL DISTRICT IN DELTA."

—RAJEEV VAIDYANATHAN

AS SUMMER TEMPERATURES climbed into the 90s in Tulare County, the CDPR proceeded with its regulatory review for Oxitec's permit application. The department hasn't made public a timeline for its decision, only that the review process will take at least several months. But while it waits, Oxitec is building a research facility in Visalia and hiring field and lab techs. DMVCD, in its collaborative role, has been supplying historical local mosquito data to the company and directing residents to Oxitec's information.

The long game for Oxitec's US trials is to gather as much data as possible to turn in to the EPA with an application for product registration and eventually market the genetically edited bugs to mosquito control districts and consumers. If it does gain that approval, however, Oxitec will likely face competition.

MosquitoMate, a company based in Lexington, Kentucky, produces a lab-created strain of male *Aedes albopictus*, infected with a species of the naturally occurring bacteria Wolbachia. When these males mate in the wild with females of the species, which can carry dengue, chikungunya, and Zika viruses, the eggs that are produced don't hatch. A large-scale trial in 2018 in Fresno County, north of Tulare County, showed a 95 percent reduction in targeted populations. These bacteria-laden mosquitoes are currently priced between $699 and $1,199 depending on the size and type of property.

UC San Diego's Omar Akbari and his team are using the genetic-engineering tool CRISPR to try to create a sterile male *Ae. aegypti*. They've done surveys, online focus groups, and interviews to better understand the public's reaction to emerging genetic technologies applied to mosquito control. Responses were across the board, he says, but it was grounding to hear concerns over the release of an organism

that researchers might not be able to control. "We need to take these concerns into our designs," Akbari says.

Oxitec, with its genetically modified mosquitoes, he says, "is in a way paving the yellow brick road. They're first to market, they're going to deal with all these difficult things, but as long as they're successful, it makes space for the next technology that might be better than Oxitec's."

DMVCD head Debboun says he'd be interested in adopting Wolbachia and Oxitec's method if the data show they work—and if they are sustainable and affordable. "We're not a private organization that has a lot of money," he says. "Sometimes you do the best you can with what money you have." As the new technologies advance, he hopes they will become inexpensive enough to add to the inventory, alongside mosquitofish and larvicides. "As they come into the market and then become affordable, this is what we're going to be doing," he adds.

In the meantime, the team at DMVCD continues to monitor and battle the enemy. Bryan Ruiz is redoubling his efforts to thwart *Ae. aegypti:* Earlier this year, he was assigned to help lead a new program to educate the public on lowering the bloodsucker's population. That's why this summer he's going door to door, educating residents and doing a bit of detective work to find potential breeding sites.

As for new technologies that may be on the horizon, he says, "If you're able to implement them, then you do. But right now it's all about house to house. ... If we weren't here, it'd be worse, so I say we are making a difference."

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