Verily project releases millions of factory-reared mosquitoes

Fresno County in California has given the green light to the environmental release of millions of mosquitoes infected with a common bacterium, aimed at shrinking populations of the yellow fever mosquito *Aedes aegypti*. This type of mosquito, which first appeared in the state's Central Valley in 2013, is a disease vector for viruses such as dengue, West Nile, Zika and chikungunya (although currently none of these pathogens is circulating in the Fresno area). The 'Debug Fresno' program's *Wolbachia pipientis*-infected mosquitoes are grown in the laboratory by MosquitoMate in partnership with Alphabet-backed Verily Life Sciences. This year the project has ramped up, with thousands of bacteria-carrying mosquitoes released each day in more Fresno neighborhoods, including the city of Sanger. The aim is to surpass the 68% drop in female *A. aegypti* achieved last year, and so far, mid-season, the project has recorded a greater than 90% reduction in mosquito release areas relative to control sites, according to a Verily spokesperson. In Australia, the not-for-profit World Mosquito Program (WMP) has been running pilot trials in the city of Townsville that also use *Wolbachia*-infected mosquitoes to interrupt disease transmission (*Gates Open Res.* **2**, 36, 2018). At the World Health Organization's instigation, the program is now running larger studies in Brazil and Colombia, as well as randomized trials in Indonesia and Vietnam (*Adv. Exp. Med. Biol.* **1062**, 355–360, 2018).

A parallel endeavor pioneered by UK company Oxitec complements these efforts. Oxitec, a subsidiary of Intrexon, aims to reduce mosquito populations using lab-reared, genetically modified (GM) mosquitoes. The latter approach, termed RIDL (release of insects carrying a dominant lethal), involves the release of transgenic OX513A male mosquitoes, which are sterile as a result of a *piggyBac*-based transposon containing the tetracycline-repressible transcriptional activator, which is lethal when expressed at high levels in the absence of the antibiotic (*Nat. Biotechnol.* **26**, 725, 2008). OX513A mosquitoes have been used to successfully reduce by >80% wild mosquito populations in Grand Cayman, Brazil and Panama.

Which of these two biocontrol approaches will ultimately prevail is likely to depend on several factors. These include practical issues, such as the feasibility of rearing sufficient numbers of sterile insects in the lab and the number of cycles of sterile insect release needed to suppress wild-type populations. But other issues, such as public perception and regulatory concerns—which have traditionally dogged GM organisms—are also likely to influence adoption

Ray Wilson / Alamy Stock Photo

A. aegypti larva

Fresno County's efforts to use bacteria-carrying mosquitoes as a biopesticide began in 2017. The bacteria *Wolbachia* has been studied extensively by microbiologists and entomologists for decades and lives symbiotically within more than half of all insect species. While it is common among insects, including bees and butterflies, it cannot be transmitted to humans

Last November, the US Environmental Protection Agency (EPA) gave the biotech startup MosquitoMate, founded by medical entomologist Stephen Dobson of University of Kentucky, the go-ahead to use its *Wolbachia*-infected male mosquitoes (ZAP Males) to fight the Asian tiger mosquito (*Aedes albopictus)* in the District of Columbia and 20 US states

Wolbachia-carrying male mosquitoes are rendered sterile by the bacteria. When released into the environment, they mate with females in the wild and the eggs don't hatch, thus reducing the local mosquito population. The company sells its product to homeowners, golf courses, hotels and other customers.

In China, researchers from Sun Yat-sen University in Guangzhou, in collaboration with Michigan State University in East Lansing, are also using factory-reared *Wolbachia*-infected mosquitoes to reduce *A. albopictus* populations. Pilot studies with

Smallpox antiviral ends decades-long search

A 17-year-long saga ended in July with the first approval of a drug for treating smallpox infections. Long sought after, but particularly so after the anthrax attacks in 2011, a small-molecule inhibitor of virus extrusion from cells, Tpoxx (tecovirimat), was approved by the FDA in July, after the agency's Antimicrobial Drugs Advisory Committee voted unanimously for approval in May. SIGA Technologies of New York took the molecule through clinical trials, after they showed in 2005 that it protected mice from lethal infections with a related poxvirus. (*J. Virol.* **79,** 13139–13148). The FDA based its decision on results from mice, enabled by the 'animal rule', which kicks in when human studies are unethical. In a pivotal trial, 380 healthy people were dosed for 14 days with no severe adverse events, as reported in June (*N. Engl. J. Med.* **379,** 44–53, 2018). The government had been seeking a treatment for smallpox for decades. Vaccination programs for smallpox ended around 1979, when the virus was deemed eradicated. Hence, people below a certain age are susceptible to infection by smallpox, considered a category A bioterror agent. Although there are millions of doses of a vaccine stockpiled in the event of an infection, it cannot be given to immune-compromised people and sufferers of some skin disorders. Although all known stocks of the virus were believed to be accounted for, in 2014 researchers at the NIH discovered vials of smallpox in the institute's basement. Finally, the recent synthesis of a poxvirus from scratch has people worried that smallpox, natural or synthetic, might someday find its way into the public sphere. Tpoxx development began at the now defunct ViroPharma in collaboration with the US Army Medical Research Institute of Infectious Diseases, SIGA, and others. After the preclinical work and early safety trials, the US government ordered 1.2 million doses for the Strategic National Stockpile, garnering SIGA $500 million. Now, with the approval, SIGA will get the first Material Threat Medical Countermeasure Priority Review voucher, which could be worth many millions more.

> **"There's 1,600 trials right now with PD-1 plus something and most of them are just empirical. There's no rational basis for picking the combinations other than maybe 'this kills T cells',"** Jim Allison, from the University of Texas MD Anderson Cancer Center, explains why, with colleagues, he has created a platform using patient data and mechanistic insights to pick combinations of checkpoint inhibitors. (*BioCentury*, 19 July 2018)

their sterile male mosquitoes are taking place at sites in Guangzhou.

But for insects to work as a biopesticide requires large numbers. To ramp up production of *Wolbachia* infected *A. aegypti*, MosquitoMate teamed up with Verily. Initially, entomologists testing the approach introduced the bacteria by microinjection followed by mass rearing (*Nat. Biotechnol.* **34,** 221–222, 2016). They then separated males from females using their different sizes at the pupal stage, as males are smaller than females. With Verily's input, the entire process is mechanized and automated, from the rearing of larvae, to separation of pupae, to computer imaging algorithms to confirm that the mosquitoes for release are male. The factory can produce 1.5 million males per week, says Bradley White, Verily's Lead Scientist on the Debug Team. Vans transport male mosquitoes and distribute them across Fresno, using algorithms for GPS-controlled release to release the male insects uniformly, says Jacob Crawford, Verily Senior Scientist. Crawford adds that the company has developed tools to collect field data that allows them to monitor the effects on the wild population in "as close to real-time data collection as possible."

During 2017, the initial year of the Debug Fresno collaboration, 20 million male *Wolbachia*-infected mosquitoes were released over a 20-week period. The number of *A. aegypti* females dropped by 68% on average during the peak of the mosquito season. The initiative tracks females because only females blood-feed, thus transmitting the virus to a new host.

The WMP, launched in 2011 by its director, Scott O'Neill, also uses lab-grown *Wolbachia*-infected mosquitoes, both males and females. The program involves infecting mosquitoes in the lab with the *w*Mel strain of *Wolbachia* and is aimed at modifying—not eliminating—the wild population by allowing the bacteria to become established throughout the local population. Because this method avoids separating males from females and requires few mosquitoes—and those only initially—to infect an entire population, it is both sustainable and cost effective. Infected females are still fertile and transmit the bacteria via the egg cytoplasm, a process that is repeated down the successive generations, thus spreading *Wolbachia* throughout the local mosquito population. A *w*Mel *Wolbachia* infection inhibits replication of disease-causing viruses in *A. aegypti* mosquitoes (*Nature* **476,** 450–453, 2011).

WMP researchers are testing this *Wolbachia* strategy for reducing dengue transmission in 12 countries at sites where dengue, the world's most common mosquito-borne viral disease, is endemic. The WMP recently reported that after releasing both male and female *A. aegypti* infected with the virus-blocking *w*Mel strain of *Wolbachia* in dengue-endemic sites of Australia, Brazil, Colombia, Indonesia and Vietnam, *Wolbachia* became established in the resident *A. aegypti,* and no local dengue transmission has been observed since (*Adv. Exp. Med. Biol.* **1062**, 355, 2018). In describing the technology, O'Neill says, "We don't need to release that many mosquitoes … we set up modest production facilities in each of the countries we're working in." Once the technology is transferred for local production, the organization plays an advisory role.

Oxitec has already rolled out field studies with its GM mosquitoes in several sites around the world (*Nat. Biotechnol.* **34,** 221–222, 2016). The Abingdon-based company tailors its operations to the size of the projects for any given country. Kevin Gorman, Head of Field Operations for Oxitec, explains that eggs can be shipped anywhere in the world from the UK egg production factory, or, if an operation is very large, it might be more efficient to have an egg production plant in-country. The sorting process, emergence of mosquitoes, and dispersal of males by van are set up at each location.

As Oxitec's strategy involves genetic modification, this has sometimes proved challenging in terms of public acceptance. For example, Oxitec's proposed field trials of OX513A in the Florida Keys met some resistance from local residents (*Nat. Biotechnol.* **33**, 792–793, 2015); however, in 2016, "probably the largest referendum ever on GM insects" took place in Monroe County, Florida, according to Gorman. The result was favorable: in 31 of 33 precincts, the public voted in support of trialing Oxitec's mosquitoes. Gorman says that because the company is a trailblazer in environmental release of GM insects, "we often have to pioneer our way through the regulatory and public engagement piece in [the] countries where we work."

The patchwork of country-specific GMO regulations across the world is also a headache: "Regulatory frameworks are advanced in some countries and less so in others," says Gorman. In the United States, the Food and Drug Administration (FDA) found no significant impact on human and environmental health for Oxitec's OX513A transgenic mosquitoes; but OX513A mosquitoes have since been placed under the EPA's regulatory jurisdiction for review as a biopesticide, and the EPA has different requirements from the FDA.

"There's the regulatory piece, the public piece, and the political piece—there has to be the political will in the country to tackle the problem in the first place," Gorman says.

By contrast, MosquitoMate's *Wolbachia*-based strategy has met little resistance from the public and non-governmental organizations (NGOs) and faced fewer regulatory obstacles. Dobson says that its product has always relied very heavily on Mosquito Abatement Districts, local government bodies whose purpose is to control mosquitoes, to encourage the public's acceptance of their technology. The pathway to approval could also prove more straightforward for *Wolbachia*-based strategies than for GM mosquitoes because other microbial pesticides have already undergone regulatory review. EPA approval for ZAP Male mosquitoes, the first commercially available product based on MosquitoMate's *Wolbachia* technology, came in 2017 after a decade of working closely with the regulators at the EPA.

O'Neill says that the work of the WMP requires community consent: "We do all of our work through partnerships … We spend a lot of time on [engaging with the public], and have always had good community relationships." The WMP obtains regulatory approval for release of *Wolbachia*-infected *A. aegypti* country by country, as most have their own regulatory legislation. Importantly, however, in contrast to GM insects, there are often precedents of other biocontrol agents going through these regulatory pathways.

Other technologies, such as the CRISPR–Cas9 gene editing system, could also provide a method for gene drive strategies, which have the potential advantage that the modifications would be passed along to all progeny, thereby genetically modifying the entire mosquito population starting with only small numbers of modified insects (*Nat. Rev. Microbiol.* **16**, 509–518, 2018). Such technologies are still in development and have not matured sufficiently for field trials.

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> **"We're really focused on understanding what we need to do to ensure that, at the point of care in China, [the software is] where physician needs [it] to … provide recommendations on how to treat their patients in China. But that's going to differ from what a physician needs to have at the point of care in the United States."** Lisa Rometty, of IBM Watson Health, points out that Watson Health will now incorporate local treatment advice, following criticism that because cancer treatment recommendations are based solely on Memorial Sloan Kettering Cancer Center's experience, they are too parochial. (*STAT,* 31 July 2018).