Austral Entomology

*Austral Entomology* (2014) **53**, 363–367

*Overview*

# Rear and release: a new paradigm for dengue control

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**Abstract** Dengue continues to be the largest cause of arboviral human disease. Australia is no exception, with annual outbreaks in north Queensland. Until recently, we were restricted to measures, such as pesticide sprays and container removal, that reduce populations of the mosquito vector *Aedes aegypti* (Linnaeus, 1762). However, the Eliminate Dengue research program, an international collaboration led by Professor Scott O'Neill of Monash University, uses the bacterium *Wolbachia pipientis* to block dengue virus replication in the mosquito and reduce dengue transmission. To date, releases of adult *A. aegypti* infected with the *w*Mel strain of *Wolbachia* have been made at seven different locations near Cairns Queensland. *Wolbachia* has successfully established in each release area, with some populations having persisted for three consecutive years. Success using *w*Mel has come relatively easily, with fixation (>90% mosquitoes infected) obtained after 3 months of weekly releases of adult mosquitoes that required no concurrent vector control. We have not had success establishing *w*MelPop strain, due to the high fitness costs of this more virulent strain of *Wolbachia* to its mosquito host. New release strategies of *w*Mel that allow us to simply place egg papers in rearing buckets in the field are also showing promise. Going forward, we hope to scale up the program so that large urban areas can be treated with a minimal number of releases and labour. Finally, what evidence is there that *Wolbachia* actually prevents dengue in human populations? The definitive studies will be taking place in dengue endemic countries such as Indonesia and Vietnam where sufficient transmission allows for carefully controlled trials, although dengue incidence in *Wolbachia* treated areas of Queensland will be watched with interest.

**Key words** *Aedes aegypti*, biological control, *Wolbachia.*

## SPRAY 'EM AND SLAY 'EM: THE OLD PARADIGM OF DENGUE CONTROL

I began my career killing mosquitoes as a driver of a 1974 El Camino pickup fitted with a Leco ULV fogger, spraying the parklands near Ames, Iowa, where I was enrolled as an entomology student at Iowa State University. From there, I was hired as an entomologist with Collier Mosquito Control District in Naples, Florida. We killed mosquitoes in a big way. Using a fleet of four DC3s flying at tree-top height, we laid down a thick fog of insecticide (Fenthion®, an organophosphate) to kill the hordes of saltmarsh mosquitoes that invaded the region from the nearby Ten Thousand Islands. Finally, I became the Director of the Medical Entomology division of the Tropical Public Health Unit, Queensland Health, in Cairns. Again, we used pesticides to control mosquitoes, in this case *Aedes aegypti*, the mosquito vector of dengue.

I introduce this story with a personal history of my mosquito-spraying career to highlight that the use of pesticides is still the primary method used to control arboviruses. And this includes dengue. As there is no available vaccine, insecticides are the key weapon used to kill *A. aegypti* and control dengue. That is changing though. A new paradigm that I term 'Rear and release' is being developed and tested for the control of dengue. And as the name implies, instead of spraying to kill mosquitoes and prevent dengue transmission, we will rear and release mosquitoes. Much of the critical research is occurring in the north Queensland city of Cairns where dengue occurs annually.

### A primer on *Aedes aegypti*

The mosquito *A. aegypti* is not native to Australia. Indeed, it has hitchhiked around the globe on ships from its ancestral home in Africa in the 16–18th centuries (Tabachnick 1991). This mosquito is unusual in that it is highly domesticated. The eggs and larvae reside in artificial containers, such as buckets, tyres, pet bowls and, in the case of old sailing vessels, in wooden water storage barrels. The adult mosquito prefers to live inside dark, quiet areas within houses, buildings and even boats where it stealthily feeds on human blood. Thus the tight linkage between artificial larval habitat and the necessity to reside with and feed on humans has ensured that this 'cockroach of mosquitoes' has become a regular travelling companion as commerce spread around the globe.

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doi:10.1111/aen.12127

The strong feeding preference for humans has also enhanced the capacity for *A. aegypti* to spread human diseases. The arboviruses that cause dengue and yellow fever are, in their most prolific manifestations as urban epidemics, carried almost strictly by *A. aegypti*, with humans, the sole host. Thus, a perfect transmission cycle from *A. aegypti* to human and back maximises the amplification of the virus. During an explosive outbreak of dengue in Cairns, a single introduction of the virus in November 2008 led to an epidemic with over 100 cases per week by February 2009 (Ritchie *et al*. 2013)!

### House to house combat: traditional dengue control

The famous medical entomologist Paul Reiter said 'you can't kill a mosquito in a closet with an airplane'. He was referring to *A. aegypti* and dengue control. Because many mosquito control programs have relied upon vehicle-mounted foggers to kill adult mosquitoes, using vehicles ranging from trucks and mopeds to helicopters and airplanes, it is a logical assumption that the big guns will kill dengue mosquitoes too. However, in many tropical cities, housing consists of small, cramped houses with few rooms, small windows and poor ventilation. The female *A. aegypti* hides under beds and tables, in wardrobes and laundry piles, where it is poorly exposed to insecticidal sprays administered from trucks or planes (Perich *et al*. 2000), resulting in little impact on dengue transmission (Reiter & Gubler 1997).

The dengue control administrators in north Queensland, appreciating the wisdom of Reiter, developed a dengue control program centred on interior residual spraying (IRS). The Dengue Action Response Team, a 'SWAT' team of highly trained vector control officers, enter houses (with permission of residents) with suspected dengue activity, and spray the dark nooks and crannies where *A. aegypti* are harboured (Ritchie *et al*. 2002). Dark objects such as suitcases, quiet spaces under beds and tables, and the insides of wardrobes are treated with a dilute water-based mix of synthetic pyrethroid insecticides that kill adult *A. aegypti* for several weeks. In the yards, water-holding containers that could breed hundreds of larvae are removed, tipped over or treated with residual pellets of the insect growth-regulator methoprene. This guerrilla warfare approach is effective (Vazquez-Prokopec *et al*. 2010) but laborious, requiring several dedicated staff. IRS is also intrusive, requiring staff to spray within the private confines of the public's castle. Often, we did not hear about dengue transmission until the outbreak was well underway; these delays of notification are almost always the cause of our large outbreaks such as those in 2003 and 2008 (Vazquez-Prokopec *et al*. 2010; Ritchie *et al*. 2013).

## 'REAR AND RELEASE': A NEW PARADIGM IN DENGUE CONTROL

The concept of the rearing and releasing large numbers of insects that mate with and induce sterility in pest insects is not new. In the 1950s, massive numbers of screw worm flies (*Cochliomyia hominivorax*), a pest that caused extensive injuries and death in cattle in Florida and much of Latin America, were successfully eradicated in Florida by releases of male flies that had been sterilised by radiation. This sterile insect technique has been used, in a limited way, to control mosquitoes, and is still used today to control many pests (Alphey *et al*. 2010). However, we have developed a new biological control method, using the bacterium *Wolbachia pipientis pipientis* (a species of *Wolbachia* found in some mosquitoes but not in *A. aegypti*), that uses only limited releases of infected mosquitoes that quickly establish the bacterium in wild populations of *A. aegypti* (McGraw & O'Neill 2013).

## *WOLBACHIA*: A BACTERIA THAT 'VACCINATES' DENGUE VECTORS

The bacterium *Wolbachia* has long been of interest to biologists. *Wolbachia* infections, especially studied in vinegar flies such as *Drosophila*, induce often amazing physiological responses in their insect hosts. Sex manipulation (feminisation), parthenogenesis, male killing and sperm–egg incompatibility are observed in *Wolbachia*-infected arthropods (Werren *et al*. 2008). Of interest to mosquito biologists is the ability for *Wolbachia* to be driven rapidly into populations of uninfected mosquitoes by a process termed cytoplasmic incompatibility (CI) (for reviews, see Iturbe-Ormaetxe *et al*. (2011) and Turelli (2010)). In CI, the offspring (eggs) of uninfected females mated to *Wolbachia*-infected males are inviable and die. The reciprocal cross of uninfected males mated with infected females produce viable *Wolbachia*-infected offspring. Thus, a release of *Wolbachia*-infected male and female mosquitoes produces *Wolbachia*-infected offspring at a selective advantage, assuming no mating bias, strong CI and limited fitness costs to the *Wolbachia*-infected offspring. Thus, *Wolbachia* can spread naturally beyond the initial mating of released mosquitoes, ultimately becoming fixed in populations with infection rates approaching 100% (Turelli 2010). This drive mechanism also ensures that *Wolbachia* can persist in populations over many generations.

Professor Scott O'Neill realised that the drive mechanism of *Wolbachia* could be used to control dengue transmission in *A. aegypti*. Initially, we used a virulent strain of *Wolbachia* (*w*MelPop or 'popcorn', so named as the impact of replicating *Wolbachia* on infected cells was reminiscent of that of popcorn in a stovetop container) that is known to over-replicate in host's cells and cause premature death in *Drosophila* (McMeniman *et al*. 2009). *w*MelPop was introduced into *A. aegypti* by microinjecting the bacterium into eggs of the mosquito (McMeniman *et al*. 2009). This was no small feat, with 10 000 microinjections required to obtain two infected lines. These mosquitoes were then observed to die prematurely, indeed most died before they would have been able to transmit dengue (*ca*. 10 days after blood feeding). The concept was simple: Rear and release *Wolbachia*-infected *A. aegypti* and let the CI drive mechanism infect

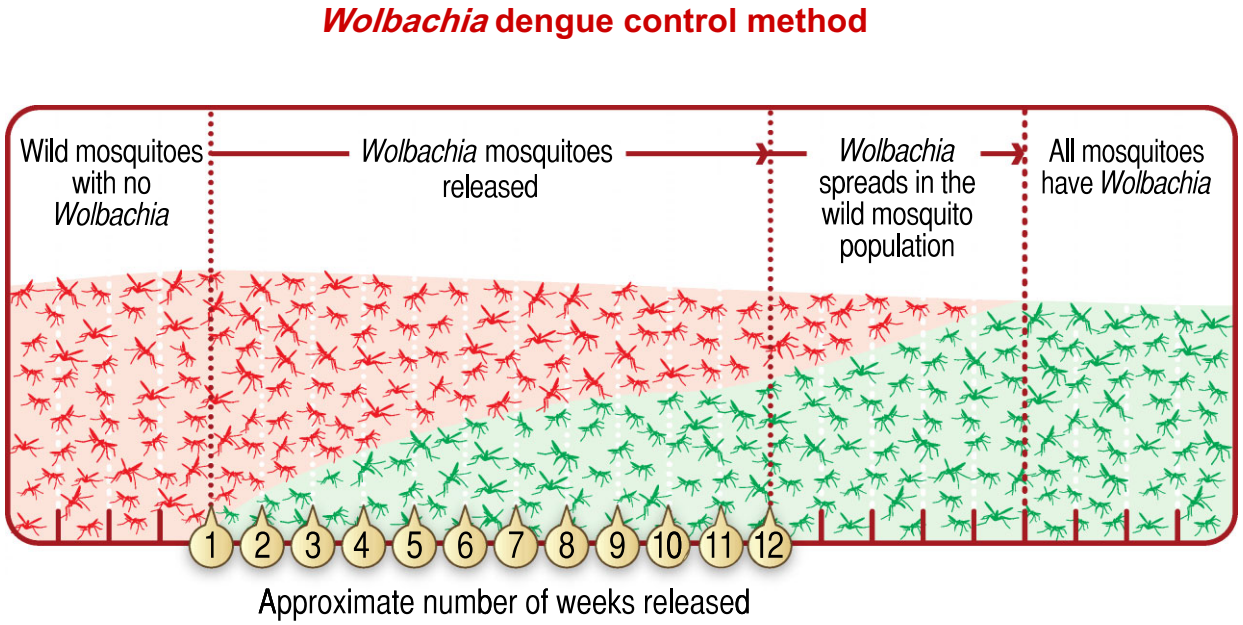


***Fig. 1.*** Cartoon of 'rear and release' Wolbachia invasion strategy. Diagram courtesy of Eliminate Dengue (http://www.eliminatedengue.com).

the population of wild uninfected *A. aegypti* with the bacterium (Fig. 1). Mosquitoes infected with popcorn *Wolbachia* would not live long enough to transmit dengue and, potentially, other arboviruses (McMeniman *et al*. 2009; Iturbe-Ormaetxe *et al*. 2011). While some dengue transmission may occur, large epidemics that have recently plagued north Queensland would be unlikely.

## Proof of concept in semi-field cages

To confirm that weekly releases of *Wolbachia*-infected *A. aegypti* would result in fixation of *Wolbachia* in a population of mosquitoes, we have conducted trials in secure large semi-field cages at James Cook University in Cairns. In these studies, fixation was obtained with both *w*Mel and *w*MelPop, although the fitness issues of *w*MelPop slowed the rate of fixation for this strain (Walker *et al*. 2011). We were now ready for open field releases, choosing to go with *w*Mel to maximise our chances of success.

## Dengue blocking: an even better outcome than life shortening

We noticed that *A. aegypti* infected with *w*MelPop were doing more than dying before they could transmit the virus – they were not even getting infected with the virus! The *w*MelPop-infected *A. aegypti* had levels of dengue infection, as measured by PCR, that were up to six orders of magnitude lower than wild *A. aegypti*, and assays indicated almost no disseminated dengue infection in the mosquito legs, a proxy transmission of the virus (Moreira *et al*. 2009). We then investigated the virus blocking capacity of *w*Mel, a less virulent strain of *Wolbachia*. Popcorn-infected *A. aegypti* had several fitness issues in addition to shortened adult lifespan, including reduced blood feeding (Turley *et al*. 2009), lower fecundity, (Yeap *et al*. 2011) and reduced egg longevity (McMeniman & O'Neill 2010). These fitness constraints could reduce the chance of establishment despite the CI drive mechanism. The less virulent *w*Mel strain does not induce these pathological effects on the host, and should be easier to establish within wild mosquito populations than *w*MelPop. Dengue blocking was also observed in *w*Mel, although not as complete as in popcorn. It is postulated that *Wolbachia* competes with the growth of dengue virus at the cellular level, and *w*Mel has lower *Wolbachia* infection loads than *w*MelPop. Nonetheless, vector competence experiments demonstrated that significant decrease in disseminated dengue infection occurred in Cairns mosquitoes infected with *w*Mel (Walker *et al*. 2011).

## Only with community support: extensive engagement

While the *Wolbachia* dengue control strategy would ultimately target urban areas of SE Asia and South America where dengue is endemic, the initial releases were planned for the Cairns region in north Queensland. Indeed, the first release of *Wolbachia*-infected *A. aegypti* was proposed for Yorkeys Knob and Gordonvale, Queensland. Gordonvale was the site where the cane toad was first released in Australia, and the irony of our decision to release there was not lost upon some residents.

Before any release began, the ED program implemented a comprehensive community engagement (CE) program in both communities, to help gauge support for a future field trial (Hoffmann *et al*. 2011; McNaughton 2012). The CE program involved development of resources (pamphlets, PowerPoint presentations, newsletters), to communicate the research to residents, and to encourage any questions and concerns, which were promptly addressed. By far, the greatest resource was

simply face-to-face communication at community events, meetings and by door-knocking. Surveys demonstrated that over 80% of Cairns residents were supportive of a release (McNaughton 2012). During the trial, regular meetings of a reference group allowed valuable two-way communication between the project and community representatives.

### Open field releases: the ultimate test

Open field releases of the *w*Mel-infected *A. aegypti* were planned for the wet season of 2010–2011. We had approval from the Australian Pesticide and Veterinary Medicine Authority, as well as the biosafety committees of the University of Queensland and James Cook University, and a risk assessment conducted by CSIRO was supportive (De Barro *et al*. 2011). In addition, critically, we had approval from the majority of local residents in the release sites. Rearing and releasing mosquitoes sounds simple, but there was considerable logistical issues that needed sorting out. Mosquitoes were reared within the large semi-field cages at James Cook University, and fed on humans to mimic natural mosquito production as closely as possible. To avoid accidentally infecting the mosquitoes with dengue, all volunteer blood feeders were tested for fever. Those febrile or even living or returning from an area with active dengue transmission were excluded. Beginning the first week of January 2011, James Cook University reared *ca*. 15 000 mosquitoes for release in the field sites. While this sounds like a lot, it only amounted to 15–20 mosquitoes per house, half of which were males that do not bite. Estimates of adult mosquito populations indicate that we were releasing numbers comparable with existing populations. Releases were as simple as possible. Once a week, a white 'Eliminate Dengue' van stopped at every 4th house, and out jumped a worker who released a cup of mosquitoes at the curbside.

The frequency of *Wolbachia* infection was closely monitored in eggs laid by mosquitoes in oviposition traps ('ovitsaps'). After the initial two-weekly releases, the infection rate in eggs collected from ovitraps was approximately 15%, representing eggs laid by females from the first week's release. But after that, as the CI drive mechanism kicked in, the frequency of *w*Mel-infected mosquitoes steadily rose until after 10 weeks, the frequency was nearly 80% despite a week-long interruption at Gordonvale due to Cyclone Yasi (Hoffmann *et al*. 2011). Simulation models by Michael Turelli of the University of California at Davis indicated that with the CI drive, we could be confident that the *w*Mel would soon be fixed even if we ceased releasing (Turelli 2010). So, after 10 weeks of releases, in the middle of March 2011, the rear and release of *w*Mel-infected *A. aegypti* ended. And, as the model predicted, the infection rate of *w*Mel continued to increase until over 90% of the mosquitoes were infected only a month later. Monitoring of the two sites has confirmed that *w*Mel has remained fixed in the population since the releases 3 years ago.

### Future work

The initial releases of *w*Mel in the Cairns region used communities that were geographically isolated to ensure the bacterium would not spread beyond the target area. However, theory indicates that the CI drive mechanism can potentially create a wave of infection (termed a Bartonian wave in reference to its descriptor Nick Barton) that could result in self-propagating spread that could eventually infect a large urban population of *A. aegypti* (Barton & Turelli 2011). So, in 2013, releases of *w*Mel were conducted in the Cairns suburbs of Parramatta Park, Edge Hill/Whitfield and Westcourt. *Wolbachia* frequencies have reached 80–90% in the three release areas, and we are monitoring adjacent areas for evidence of spread. Releases are also planned for Townville in 2014.

The Eliminate Dengue project involves many collaborators from countries where dengue is an annual public health issue. Releases of *Wolbachia*-infected *A. aegypti* are currently taking place in Vietnam, and are planned for Brazil, Indonesia and Colombia (http://www.eliminatedengue.com). The north Queensland urban areas of Cairns and Townsville will continue to serve as a leading centre of activity to analyse and optimise the *Wolbachia* rear and release strategy. We are the only locale with established populations of *Wolbachia*-infected *A. aegypti*. The evolution of both *Wolbachia* and *A. aegypti* will be monitored closely in this natural experiment. We are also testing novel ways of releasing, including the use of egg release containers where cloth strips containing infected eggs are reared in buckets in the field, and have developed new power-free traps for the inexpensive collection of *A. aegypti* (Ritchie *et al*. 2014).

Most importantly, we are closely monitoring the impact of *w*Mel infection on the incidence of dengue transmission. Large-scale releases are planned for Indonesia, Vietnam and Brazil, countries where dengue is endemic and where sufficient outbreaks occur that a formal assessment of the epidemiological protection from dengue *Wolbachia* is possible. Yet even the incidence of dengue in Cairns will provide some clues of the efficacy of the approach. Currently, there is a large outbreak of DENV-1 in central Cairns. The incidence of dengue in the Cairns region will be watched with greater than normal anticipation the next few years.

## ACKNOWLEDGEMENTS

I thank Roger Kitching for inviting me to submit this story, Helen Cook and Peter Cook of Eliminate Dengue for input, and Peter Ryan (Eliminate Dengue) for the cartoon depicting a *Wolbachia* intervention.

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*Accepted for publication 26 August 2014.*