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EMERGING TECHNOLOGIES FOR CONTROL OF *AEDES AEGYPTI* AND *AEDES ALBOPICTUS* (DIPTERA, CULICIDAE)

JULIAN ENTWISTLE
Xenex Associates Ltd., Chapel House, Barns Green, Horsham,
West Sussex, RH13 OPR, United Kingdom

Abstract *Aedes aegypti* and *Aedes albopictus* are key vectors of dengue and chikungunya. The status of the main emerging control technologies for these species which have reached open cage or field trial stages is reviewed: insecticide impregnated fabrics, lethal ovitraps, *Mesocyclops*, *Wolbachia*, densovirus and Release of Insects Carrying a Dominant Lethal (RIDL) Gene. These technologies have shown effectiveness, with insecticide impregnated fabrics being the next addition to large scale programmes and RIDL the most promising alternative approach to insecticide treatments. RIDL enables control of both species where a proportion of the mosquito resting sites and breeding sites cannot be sprayed. It has proved effective in cage trials and in a first open field trial where it led to a reduction of 80% in the population of wild *Ae. aegypti*.
Key Words Dengue, control technologies, RIDL, insecticide impregnated fabrics, lethal ovitraps, *Mesocyclops*, *Wolbachia*, densovirus

INTRODUCTION

The purpose of this paper is to review recent information on emerging technologies for control of *Aedes aegypti* (L.) and *Aedes albopictus* (Skuse) and to consider the potential of these technologies to be adopted widely by control programmes.

The main diseases transmitted by *Ae. aegypti* and *Ae. albopictus* are dengue, yellow fever, chikungunya, West Nile virus and St. Louis encephalitis. *Ae. aegypti* is the principal vector of dengue and dengue is the most prevalent of these diseases with 2.5 – 3.6 billion people now at risk and up to 500 million dengue infections worldwide every year with approximately 36 million resulting in dengue fever (Beatty, 2009; WHO, 2009). The disease has spread rapidly and is now endemic in more than 100 countries in Africa, the Americas, the Eastern Mediterranean, South-east Asia and the Western Pacific. An estimated 500,000 people with DHF require hospitalisation each year, a large proportion of whom are children and about 2.5% of those affected die (WHO, 2009).

There has been a recent, dramatic increase the geographical range of *Aedes* mosquitoes and associated disease problems. For example, in the last 50 years the worldwide incidence of dengue has increased 30-fold (WHO, 2011). Urbanisation, international travel and the movement of used car tyres appear to account for the recent expansion of the range of these mosquito species and associated diseases. Climate change may also be a contributing factor and may continue to drive range expansion.

Apart from the vaccine for yellow fever, no vaccines or drugs are available for these diseases and therefore their control depends on interrupting disease transmission through control of the mosquito vectors. Traditional methods rely on space spraying for control of the adults and larviciding and source-reduction for control of the larvae. Weaknesses of these techniques include the lack of persistence of space-sprays and their inability to reach many of the mosquito resting sites, particularly indoors. The increased incidence of the diseases and increased range of the mosquitoes demonstrate that the traditional methods do not offer a complete solution and there is a need to identify and evaluate new control technologies.

The alternative and emerging control technologies considered in this paper are insecticide impregnated fabrics, lethal ovitraps, *Mesocyclops*, *Wolbachia*, densovirus and the Release of Insects Carrying a Dominant Lethal (RIDL) gene. All these techniques have reached large cage trial or open field trial stage and some are used in practical control programmes. The principal studies on their effectiveness are summarised and then the future prospects for their widespread application are considered in the Discussion.

RELEASE OF INSECTS CARRYING A DOMINANT LETHAL

RIDL is based on the Sterile Insect Technique (SIT) which is a species-specific, environmentally friendly and proven method of pest control based on the mass-release of sterile insects. SIT has been used successfully for decades to control several agricultural pest insects (Dyck et al., 2005). Several trials in the 1970s and 1980s highlighted the potential of SIT to be applied to mosquito control, however SIT is not currently in operational use against mosquitoes (Alphey et al., 2009; Benedict and Robinson, 2003). This is mainly due to the damaging effect of sterilising doses of radiation which makes irradiated male mosquitoes less able to compete for mates (Alphey et al., 2009; Benedict and Robinson, 2003; Helinski et al., 2006). A genetics-based approach termed RIDL® removes the need for radiation-sterilisation, making the sterile-male methods an attractive option for mosquito control (Alphey and Andreasen, 2002; Alphey et al., 2009; Fu et al., 2010; Phuc et al., 2007). RIDL strains have been constructed in *Ae. aegypti* and *Ae. albopictus* and are currently in contained and open field trials (Fu et al., 2010; Labbe et al., 2010; Phuc et al., 2007).

OX513A is an engineered strain of Ae. aegypti that has been through extensive laboratory testing and open field trials (Kongmee et al., 2010; Phuc et al., 2007). This strain has been imported into several countries and has been used in open release experiments in the Cayman Islands, Malaysia and Brazil (Luke Alphey, pers. comm.). Large cage suppression trials in 2009 were successful in eliminating *Ae. aegypti* using the female-flightless strain OX3604C (Wise de Valdez et al., 2011). Six large cages of between 232 cubic feet to 504 cubic feet each containing a stable mosquito population were split into 3 treated and 3 control groups. RIDL males were added once a week at 10 times the number of wild type males present in the cages. Successful elimination was achieved in all of the treated cages after 20 weeks of treatment.

The first ever release of the RIDL system was performed in 2009 in the Cayman Islands (Harris et al., 2010). This study showed that RIDL males were able to compete successfully for females against wild males in the field. A suppression trial in 2010 in the Cayman Islands using OX513A demonstrated the ability of this strain to suppress a population in the field (Harris et al., 2010). The open field trial reduced a population of *Ae. aegypti* by 80% compared to control sites over approximately 4 months of releases of over 3 million RIDL males. This study showed that RIDL can be successfully used in the field to control *Ae. aegypti*.

INSECTICIDE IMPREGNATED FABRICS

In cluster randomised trials in Trujillo, Venezuela and in Veracruz, Mexico, Kroeger et al. (2006) demonstrated that insecticide treated curtains and insecticide treated water container covers led to a reduction of 71-88% in the abundance (Breteau Index) of *Ae. aegypti* in treated homes and 50-88% in untreated homes. In contrast, in nearby communities not in the trial the entomological indices followed the rainfall pattern and showed no overall decrease. A serological study in Trujillo further suggested that the intervention significantly affected dengue transmission.

In six villages in a peri-urban area of Cambodia, Seng et al. (2008) incorporated long-lasting insecticidal netting treated with deltamethrin into the design of the covers for water containers, the main breeding sites for *Ae. aegypti*. Their effect on immature and adult female populations of this mosquito was compared with populations in six nearby control villages before and for 22 weeks after distribution of the jar covers. There were significantly fewer pupae per house in intervention villages than in control villages (6.6 and 31.9, respectively, $p<0.01$). Two weeks after the intervention, the average number of indoor resting female *Ae. aegypti* per house in the intervention villages had declined approximately three-fold, whereas in the controls there was only a slight reduction (16%). The magnitude of the difference between the two areas diminished over time, which contact bioassays confirmed was likely due to a gradual reduction of insecticidal effect of the jar covers.

In a cluster-randomised trial with permethrin impregnated bednets in Haiti, Lenhart et al. (2008) found that *Ae. aegypti* abundance fell in homes using the bednets, with Breteau Index reduced by 88%. Control houses located within 50m of a bednet house had significantly lower Container Index and Pupae per Person, an effect that extended to 100 m by 5 months. An IgM serosurvey showed a 15% decrease in the number of IgM-positive individuals from baseline to12 months later. So, insecticide-treated bednets had an immediate effect on dengue vector populations after their introduction, and over the next 5-12 months, the presence of insecticide treated nets may have continued to affect vector populations and dengue transmission.

LETHAL OVITRAPS

The standard ovitrap used for monitoring purposes was first modified by K. L. Chan to prevent the emergence of larvae as a supplementary measure for the control of *Ae. aegypti* in the Singapore Paya Lebar International Airport (Chan, 1972) and it proved to be so efficient that *Ae. aegypti* were eradicated from the airport one year after its introduction. Chan developed this ‘autocidal’ trap further to maximise attractiveness to the egg laying female *Ae. aegypti* and to prevent emergence of adults from eggs laid in the trap (Chan et al., 1977).

Zeichner and Perich (1999) adapted this further to create a lethal ovitrap (LO) with the egg-laying strip in the trap treated with insecticide, thereby killing the adult female mosquitoes attracted to the trap as well as any eggs they laid. Perich at al. (2003) tested the effect of 5 lethal ovitraps indoors plus 5 outdoors for each of 30 houses in each of two Brazilian municipalities: Areia Branca and Nilopolis in the State of Rio de Janeiro. They found that *Ae. aegypti* were significantly reduced in terms of the number of positive containers (4-5 vs. 10-18) and pupae/house (0.3-0.7 vs. 8-10) in treated vs. untreated houses, 3 months post treatment. The numbers of *Ae. aegypti* females resting indoors were reduced in Areia Blanca but not Niloplis.

Sithiprasasna et al. (2003) evaluated a LO for the control of *Ae. aegypti* in three villages in Ratchaburi Province, Thailand in 1999 and 2000. Two blocks of 50 houses (a minimum of 250 m apart) served as treatment and control sites in each village, with each house in the treatment area receiving 10 LOs. In 1999, the LO had a negligible impact on all measures of *Ae. aegypti* abundance that were assessed; however, fungal contamination of insecticide-impregnated strips may have been responsible for the low efficacy. In 2000, significant suppression was achieved based on changes in multiple entomologic criteria (containers with larvae, containers with pupae, and number of adult *Ae. aegypti*); however, control was not absolute and neither immature nor adult *Ae. aegypti* were ever eliminated completely, primarily due to the high number of alternative oviposition sites.

Ritchie et al. (2009) and Rapley et al. (2009) evaluated the effect of deployment of approximately four LOs per household in Cairns, Queensland, Australia, over a four-week period in the wet and dry seasons. In the dry season they found that no significant decrease in female *Ae. aegypti* could be attributed to the LO. However, in the wet season the mean number of female *Ae. aegypti* collected after 4 weeks was significantly less than the control. The LOs can harbour mosquitoes after the insecticide has lost its activity and therefore biodegradable LOs, made of plasticised amylose maize polymers that slowly degrade upon contact with water, were also tested in the wet season and led to a reduction in *Ae. aegypti* measured by BG-Sentinel traps but not by sticky traps.

MESOCYCLOPS

Predatory *Mesocyclops* copepods are known to kill *Aedes* larvae and have been used successfully in practical control programmes in Vietnam. For example, Kay et al. (2002) report on remarkable success in controlling *Ae. aegypti* and *Ae. albopictus* in 6 communes with 11,675 households and 49,647 people in the northern provinces of Haiphong, Hung Yen, and Nam Dinh in Vietnam, using local *Mesocyclops* (mainly *M. woutersi* Van de Velde, *M. aspericornis* (Daday) and *M. thermocyclopoides* Harada). This was achieved through an integrated community participation programme also involving the removal of small containers unsuitable for seeding with *Mesocyclops*. The outcome was at least 86% reduction in *Aedes* and complete eradication from several of the communities. However, variable dengue incidence rates made the clinical and serological comparison of control and untreated communes problematic.

Marten (1990) tested *Mesocyclops albidus* (Jur.) for the control of *Ae. albopictus* in stacks of discarded tyres near New Orleans, and found that the larvae of these mosquitoes virtually disappeared within two months and adults disappeared about one month later and remained scarce for at least another year.

Wolbachia

Introducing endosymbiotic Wolbachia bacteria to the larval stage of wild populations of *Ae. aegypti* can shorten the adult mosquito lifespan, thereby reducing its ability to transmit dengue. For example the ‘Popcorn’ strain of *Wolbachia* approximately halves the adult life of its insect host in the laboratory to a median of about 25 days (McMeniman et al., 2009) and this shorter lifespan would reduce but not eliminate the transmission of dengue viruses as they need an extrinsic incubation period of 12 – 14 days in the vector before being transmitted to humans (Murphy et al., 2010). *Wolbachia* can occur naturally in *Ae. albopictus* but not *Ae. aegypti*. Strains from *Ae. albopictus* have been used to infect *Ae. aegypti* (Ruang-areerate and Kittayapong, 2006) and have been demonstrated to cause cytoplasmic incompatibility, with an increase in the number of eggs laid by infected females. *Wolbachia* can be transmitted transovarially and therefore the trait propagates into the next generation. Models show that the *Wolbachia* infected strain can displace the natural strain over several generations, so the mosquito population is rendered harmless in terms of transmission of dengue.

An open field trial of the *Wolbachia* method for dengue control recently started in the Cairns region of Queensland (O’Neill, 2010) and following an initial release of 6,000 *Wolbachia* infected *Ae. aegypti* in January, with weekly releases at two locations for a 12-week period, it was reported on 17 February 2010 that mosquitoes with the *Wolbachia* bacteria already made up 20% of the population (Petrinec, 2011).

Densovirus

The *Aedes densonucleosis* virus (AeDNV) is a naturally occurring mosquito virus in *Ae. aegypti* and *Ae. albopictus* with which larvae can be infected by adding virus to their water. Suchman et al. (2005) reported that larvae are killed in a dose dependent manner, and sublethal doses cause a delay in pupation and premature death of the adults with a significant fraction dying before the extrinsic incubation period for dengue virus is completed. A Leslie-Lewis model predicted that AeDNV infection potentially reduces vector capacity by ~77%.

Suchman et al. (2005) also undertook population cage studies. Two containers of water were placed in the cages: one served as the larval habitat and the other was the oviposition site. AeDNV was introduced into the larval habitat containers in infected cages at a concentration too low to cause significant larval mortality. Over a period of several weeks the virus concentration increased in the larval habitat container and virus was detected in the oviposition site container. Subsequently the number of eggs laid in the oviposition sites of the infected cages became significantly lower than in the uninfected control cages. These results suggest that virus can accumulate and persist in a larval rearing site to concentrations that affect the lifespan and vectorial capacity of the mosquitoes and that it can be spread to new larval rearing sites by vertical transmission.

Wise de Valdez et al. (2010) undertook large cage experiments which showed that adult female *Ae. aegypti* oviposition behavior led to successful viral dispersal from treated to untreated oviposition sites but that the AeDNV titers reached were not sufficient to reduce egg densities.

DISCUSSION

All of the emerging technologies described have been shown to have an effect on *Aedes* abundance. Most of the work has been undertaken with *Ae. aegypti*.

Of the technologies considered, control by densovirus is the only one not yet tested in an open field trial. The results obtained with it in cage trials are mixed and more demonstrations of its ability to deliver a practical control effect will be required before this could be considered a realistic candidate for incorporation in practical control programmes.

At the other end of the spectrum, *Mesocyclops* has been successfully demonstrated in Vietnam on a relatively large scale. However, its success depends on having a small number of identifiable sources of the mosquito larvae per household and the thorough destruction of other breeding sites, a labour-intensive task. The water must not be emptied and the vessels must not be cleaned otherwise the *Mesocyclops* will die. There is also a question over the acceptability to consumers in many countries of copepods in their drinking water.

Lethal ovitraps are an attractive alternative to large-scale insecticide spray programmes in terms of reducing the potential for residents to be exposed to insecticide. Public acceptance of this approach has therefore been relatively good (Ritchie et al., 2009). However, the results in terms of reducing *Ae. aegypti* abundance have been mixed. The approach suffers from the need to place and maintain several traps per household and the traps will only be effective if natural breeding sites are limited. The traps need to be attended regularly to ensure water levels and insecticide are replenished and large-scale programmes with ovitraps demand considerable manpower and repeated access to dwellings. Mass deployment of ovitraps was planned in Key West, Florida in 2010 but because of the difficulty experienced in accessing the sites in which staff were working and the extensive labour involved, it was decided to reduce the number of traps from 15,000 to 5,300 (Florida Keys Mosquito News, 2010). In the Philippines, a programme is planned in 2011 to distribute 200,000 kits of mosquito ovicidal-larvicidal traps to communities nationwide (Philippine Information Agency, 2011). Evaluation reports on the efficacy of the traps in Florida and the Philippines will help to determine whether future large-scale deployment of this control method is advisable.

The evidence for the effectiveness of insecticidal impregnated materials appears strong and although sleeping under mosquito nets is less relevant for protection against dengue than malaria (*Ae. aegypti* actively feeds during the day), the treatment of curtains, window netting, water vessel covers and other potential resting sites appears well justified. However, care will need to be taken to avoid using similar classes of chemical to larviciding and space-spraying, in order to delay the development of resistance to the insecticides.

Wolbachia is attractive in offering the potential for displacement of a native population capable of transmitting dengue with an introduced strain that is less capable of doing so, therefore potentially offering a long-term solution once the displacement has occurred. However, the ability in practice to compete successfully with a native wild, uninfected strain remains to be demonstrated as does the retention of the *Wolbachia* infection by *Ae. aegypti* through several generations in the natural environment. Although regulatory approval was granted in 2010 by the Australian Pesticides and Veterinary Medicines Authority to begin open field testing of the *Wolbachia* method for dengue control in the Cairns region of Queensland, Australia (O'Neill, 2010), it can be anticipated that regulatory authorities will be concerned that an introduced, *Wolbachia* infected strain would persist in the environment and therefore the full environmental impact would need to be assessed very carefully before approval as it is irreversible.

RIDL has an advantage in this respect in that only male (non-biting) mosquitoes are released and the genetic trait they carry is lethal and therefore does not persist into the next generation. As the insect itself is the vector of the control agent (the lethal gene) the technique makes use of the ability of the male mosquito to find females and does not rely on operators gaining access to residential properties or spray droplets reaching inaccessible resting sites. Hence RIDL offers a highly effective and unobtrusive delivery mechanism for the control of both *Ae. aegypti* and *Ae. albopictus* without excessive manpower being required in the field. RIDL will be at its most cost-effective when population levels are suppressed (by other control methods or natural variations in the population levels) as fewer mosquitoes need to be released to achieve population suppression. Results so far with this approach appear promising and further, larger scale pilot studies will help to demonstrate its cost-effectiveness, practicality and epidemiological impact. Open releases of the genetically modified mosquitoes have occurred in Brazil, Malaysia and the Cayman Islands and proposals for further releases are under consideration by several other countries and their regulatory authorities. Unlike *Wolbachia* and other traits, such as refractory genetic modification, the RIDL trait does not persist in the environment and therefore the evaluation of its long-term environmental impact is not of concern.

CONCLUSION

There is a major need for new technologies to supplement or substitute traditional control practices for *Ae. aegypti* and *Ae. albopictus* to combat the rapid rise in dengue, chikungunya and other diseases transmitted by these mosquitoes. The emerging technologies described in this paper have all shown some measure of effectiveness, with insecticide impregnated fabrics being the most obvious next addition to large scale programmes and RIDL the most promising alternative approach to insecticide treatments. Much needs to be done to prove the contribution of densovirus. Question marks remain over the large-scale application of lethal ovitraps and *Wolbachia* may face regulatory challenges, but once results are available from deployment of these approaches in 2010 and 2011 it will be possible to determine their prospects more accurately. No single control method can be expected to offer a complete solution and effective *Aedes* control campaigns will continue to demand careful planning, communication, community participation and the integration of a set of effective interventions.

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